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Chromatographic methods for the determination of various barbiturates: A review

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Abstract

Barbiturates are generally used in the treatment of epilepsy, convulsions, and anesthesia. Different analytical methods are used to identify, quantify, and determine barbiturates in other dosage forms. The cells of the central nervous system are highly sensitive to barbiturates. Barbiturates will enhance the GABA receptor activity and result in the inhibitory action of neurotransmitters due to the opening of chloride channels. This review summarizes the analysis of Barbiturates in Biological samples like hair, blood, serum, urine, and pharmaceutical dosage forms. Bioanalytical techniques have been used to analyze these drugs in biological samples such as blood, urine, and hair. These analytical techniques include High-performance liquid chromatography (HPLC), Gas chromatography (GC), Mass spectroscopy (MS), Thin-layer chromatography (TLC), and Ultra-violet spectroscopy (UV), Capillary electrophoresis which can produce the most appropriate results. This review gathers information, for the first time, regarding the determination of Secobarbital, Amobarbital, Pentobarbital, Mephobarbital, and Pentobarbital analytical methods for these drugs.

Keywords: Barbiturates; Analytical techniques; Drug analysis; Chromatography; Method validation**.**

1. Introduction

Barbiturates have been used for many years for the treatment of convulsions, anxiety. It can produce effects like a sedative, hypnotic and can also act as a relaxation effect on muscles. But these drugs are distributed without any prescription in many countries. It leads to drug abuse. These drugs have very narrow therapeutic index even a slight increase in the dose of barbiturate may result in serious side effects such as coma and may even lead to the death of the patient. These side effects may include bronchitis, respiratory depression, and fluctuation in the levels of blood pressure, Irregularities in the menstrual cycle, hallucinations, depression, and other mental impairments. These can interfere with both sensory and motor neurons.

Barbiturates are derived from barbituric acid. They are classified based on the duration of their action. They include long-acting, intermediate, short, and ultra-short. Oxidation occurs by various enzymes present in the liver which is the main site through the elimination of barbiturates. But during this elimination mechanism, the osmotic balance gets affected [1]. Not only has the liver it also affects the kidney and increases blood glucose levels [2]. In case of barbiturate poisoning, treatment includes providing drugs that can make urine into alkaline conditions, activated charcoal. Infants are found to be born with congenital defects if the mother takes phenobarbital during pregnancy [3]. If barbiturates are given during the C section, it can cause depression symptoms in neonates by crossing the placenta. From the past few years, the causes of death due to usage of various barbiturates are found to be increased mainly with phenobarbitone [4]. During analysis of several blood samples after post-mortem reported that many deaths are caused due to the increase of barbiturates to toxic levels due to its narrow therapeutic index levels [5]. The withdrawal symptoms of barbiturates include anxiety, vomiting, and abdominal cramps. An increase in dose above the safety concentration results in a decrease in blood pressure, cardiovascular, and renal failure. It may also cause sudden death [6]. Analysis of samples in

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the biological matrix using techniques like UV [7], GC [8, 9], LC [10, 11], HPLC, and CE are followed. But to get the information of the drug which is present at very low concentration in the biological matrix proper extraction techniques such as Solid-phase extraction, Liquid-liquid extraction, and Hollow fiber liquid micro extraction can be carried to improve detection and their identification through analytical methods [12, 13, 14, 15].

1.1. Physicochemical properties and mechanism of action

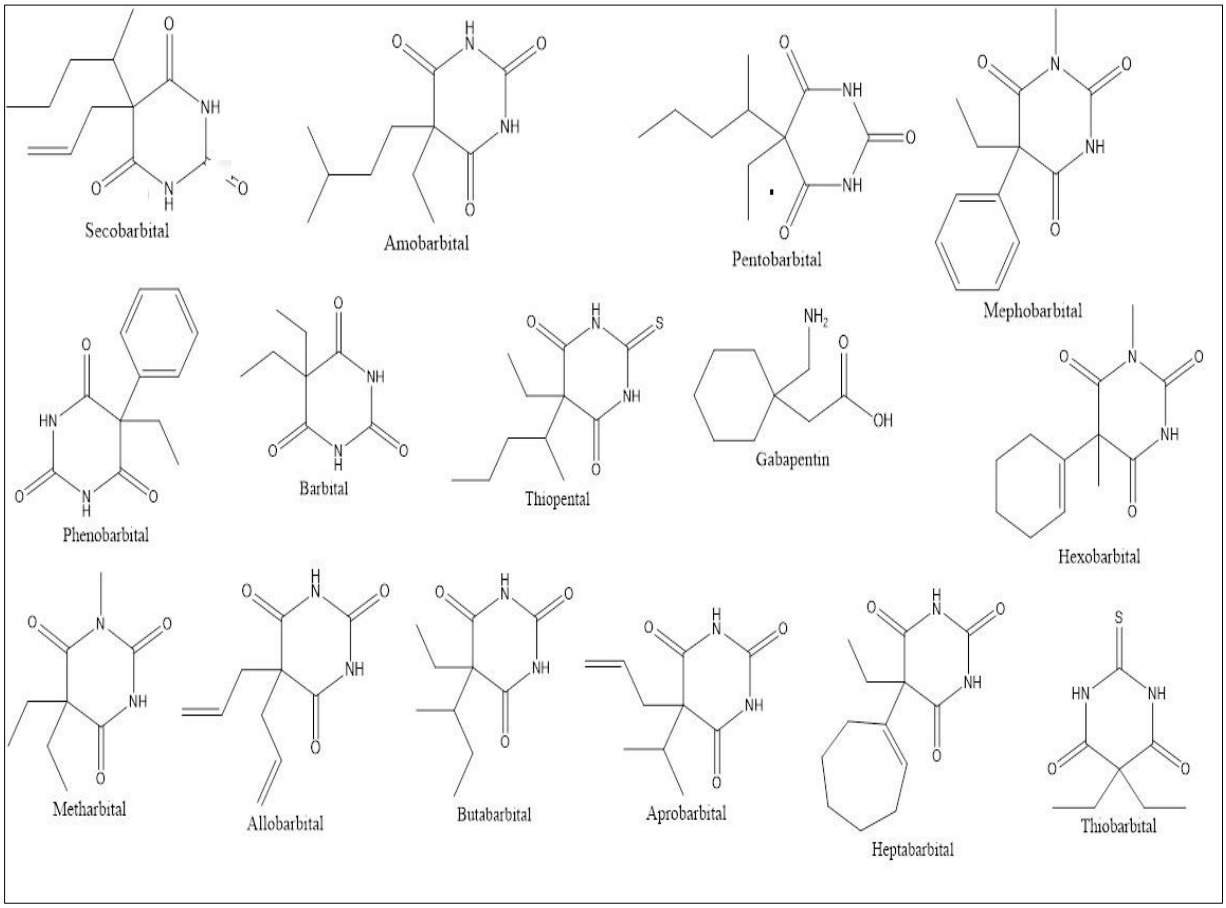

Figure 1 Overview of Chemical structure of diverse group of sedative-hypnotic drugs.

Barbiturates contain N atoms without a lone pair of electrons hence they are not basic. The acidic nature of barbiturates is higher when compared to acetic acid. The keto-enol tautomers are found in barbiturates due to their acidic nature. The solubility of drugs depends on the binding nature of the proteins. The potency and duration of the barbituric acid depend on the length of the chain at position 5 of barbituric acid. If the length of the alkyl chain increases the duration of action will decrease. The presence of sulphur at position 5 results in an increase in lipophilic nature and an increase in hypnotic activity. To produce sedative or hypnotic action the number of carbon atoms should be between 6 to 10 substituents at C 5 position. If the value is 7 to 9 the action will be faster but only for a shorter duration. The duration of action will be less if the sulphur group is at position 2 replaced with the oxygen atom. Even though the number of carbon atoms is the same the aliphatic substituents are less potent when compared to aromatic substituents. If any alkyl groups are attached to 1 or 3 positions, then acidic nature will decrease and that results in less duration of action [16]. The salts of barbiturates are highly soluble in both saline and water. If the alkaline nature of barbiturates decreases, it results in precipitation of the drug. The addition of 6 % anhydrous sodium carbonate can prevent this precipitation. Thiopentone can be refrigerated for up to 14 days and it can be stable at room temperature for up to a week. The binding of barbiturates with albumin protein can range up to 80 % but in case of any liver cirrhosis, the binding will decrease. If the drug is present as unbound, it may enter the brain through the blood-brain barrier. The main site of metabolism is the liver, due to hepatic oxidation. The phenobarbital excretion can result in an increase in alkalinity in urine. Barbiturates increase the metabolism of anticoagulants. Barbiturates will enhance the GABA receptor activity and result in the inhibitory action of neurotransmitters due to the opening of chloride channels [17]. The binding barbiturate to GABA receptors is not specific; it results in a lower therapeutic index and results in adverse reactions [18]. On regular use, the tolerance will increase which results in increasing the dose levels. Barbiturates contain 2 ethyl groups which are the same for all these drugs, but the remaining structure when replaced results in different derivatives. When 5 alkyl

groups are replaced with polar groups it reduces the hypnotic activity. If there is branching in chain isomers the hypnotic action produced will be higher, but the duration of action will be less [19].

2. Analytical methods for determination of barbiturates

2.1. Liquid chromatography

In liquid chromatography, the mobile phase is liquid. There are many advanced techniques for determining the quality and quantity of various barbiturates. These methods include High-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC). These methods have also been used for Forensic, Toxicological, and doping studies. To identify the presence of these compounds in biological samples like blood, serum, plasma, urine, hair, and some other body secretions can be collected for analysis. The presence of barbiturates like phenobarbital, amobarbital, and secobarbital in milk can be detected by LC-MS and LC-ESI-MS/MS with the electrospray ionization method. However, the presence of different proteins and fats in milk may result in difficulty in the analysis [20, 21].

Table 1 Report of Liquid chromatographic conditions for barbiturates

Drug	Matrix	Method	Column	Mobile phase	Flow rate	Detector	Reference
Phenobarbital	Plasma	RP-LC	Synergi MAX-RP C12	Water: acetonitrile: methanol (58.8:15.2:26, v/v/v), gradient	1.2 mL/min	PDA detector	20
Pentobarbital, Amobarbital	Serum	LC/ Electrospray MS	X Terra MS C18 column	Acetonitrile and ammonium acetate 10 mM, pH 7, gradient	0.15 ml/min	PDA detector	21
Methohexital	Human whole blood	LC-tandem MS	CTO-20A column C18 column	Water: Acetonitrile	1ml/min	PDA detector	24
Phenobarbital	Urine	(LC/MS/MS)	CTO-10AVP column, Isocratic	Ammonium acetate (5 mM) in 70% acetonitrile	0.3ml/min	QTRAP triple quadruple analyser	25

2.2. High-performance liquid chromatography

Reverse phase High-performance liquid chromatography is most used in the forensic and pharmaceutical analysis of barbiturates. There are a wide variety of columns but mostly octadecyl silica columns are used. Thermo responsive polymers, such as poly (N-isopropyl acrylamide), have been developed as a packing material in which the separation of the analyte of interest takes place based upon the temperature on silica beads where the process of transition takes place. Using thermo responsive HPLC compounds such as phenobarbital, and secobarbital using the thermo responsive HPLC. To improve the resolution of compounds, derivatization before injecting the sample can remove the interferences present in the sample [26].

Amobarbital has an action that can reduce the death of cells which can occur due to oxidation. To improve the stability cross-linking of amobarbital with Hyaluronic acid can be detected by UV-HPLC methods. With cross-linking the solubility and release of amobarbital in biological fluids are increased [27].

Pentobarbital has been used for producing sedative action during scanning procedures in infants. The dosage levels range from 4-8mg/kg body weight. For determination of stability studies of pentobarbital to identify the presence of various impurities, assay levels, and degradation using RP-HPLC. The degradation studies of pentobarbital were performed in presence of acid, alkali, oxidation, exposure to sunlight, temperature, and hydrolytic stress. The absorbance of pentobarbital in UV was reported at 214 nm. LOD and LOQ for pentobarbital were 2.103 and 3.979 µg/ml respectively. Degradation of pentobarbital at 1N HCL and 1N NaOH doesn't occur. Hence degradation using higher

normality levels of acid and base were performed. But oxidative degradation peak was reported at 1.44 min retention time for pentobarbital [28].

Rapid Resolution (RR) and Rapid Resolution High Throughput (RRHT) techniques have advanced methods in HPLC that can reduce the period of run time. For scalability, columns of different lengths, particle sizes are being used which can even improve the efficiency of separation of barbiturates. The particle size in the stationary phase place can be calculated based upon selectivity. Packing of a different sized particle at different dimensions within the column when there are similar characteristics in the chromatogram. At a lower flow rate, it requires more time for analysis. To improve the speed for separation and maintain efficiency, the columns which have lower-sized particles are useful. It will also reduce the high system back pressure which is a major drawback in HPLC [29].

2.3. Ultra-Performance liquid chromatography (UPLC)

For barbiturate analysis in urine new and highly sensitive methods like UPLC/MS/MS. They produce results of high throughput. They have been reported methanol is used for the dissolution of thiobarbital and phenobarbital. Urine samples are centrifuged at 13000 rpm. The signal to noise ratio was reported as less than 5:1 at LOD and the signal to noise ratio was reported as greater than 10:1 at LOQ. A large amount of sample loss occurs in GC/MS due to different extraction methods like liquid/liquid extraction and other derivatization. By using UPLC it can prevent the loss of samples and also save time. The MS produces ions by the negative electron impact ionization method [36].

2.4. Ultrafast Analysis Rapid fire with Triple Quadrupole Mass Spectrometry

Table 2 Report of HPLC analytical conditions for Barbiturates

Drug	Method	Detector	Column	Mobile phase	Injection volume	Reference
Pentobarbital	HPLC	Diode array detector	Reverse phase Nova-Pak C18 column.	(0.01 M phosphate buffer pH 3: methanol; 40:60 v/v), Gradient.	25 µL	28
Barbital, Phenobarbital, Aprobarbital. Pentobarbital.	HPLC	UV, 214 nm	ZORBAX Eclipse XDB-C18 column.	Mobile phase A methanol; mobile phase B water (50:50, A: B), Isocratic.	10 µL	30
Pentobarbital	HPLC	UV detector	Nucleosil C-18 column.	0.01 M phosphate buffer at pH 3.5: acetonitrile (72:28v/v)-gradient.	10 µL	31
Secobarbital	HPLC - MS/MS	MS detection	Agilent Eclipse Plus C18 (1.8µm 4.6 x 50 mm particle size).	Mobile phase A of 0.2% acetic acid solution, mobile phase B HPLC grade methanol. Gradient	10 µL	32
Levetiracetam	RP-HPLC	UV-Vis detector	C18 column (5 µm, 250 × 4.6 mm)	Water: acetonitrile (90:10 v/v)	40 µL	33
Thiopental	RP-HPLC	UV detector	ODS column (150 mm× 4.6 mm× 5 µm)	Potassium Dihydrogen Phosphate and methanol (40:60 v/v)	20 µL,50 µL	34
Gabapentin	RP-HPLC	UV-Vis detector	Zorbax SB C18(250 x 4.6mm), 5µm	Methanol: water (50: 50 v/v)	10 µL	35

As the rate of addiction to these drugs is increasing there is a need for the development of new methods which can produce high levels throughout the screening. By using Rapid fire triple quadrupole mass spectrometers can analyze

these barbiturates within a very short time. The sample of urine can be prepared by the spiking method; it can be further diluted. The diluted samples were kept in a micro plate and centrifuged. The samples can be analyzed by a rapid-fire triple quadruple system effectively within a short time [37].

2.5. Ultra-high-performance liquid coupled with high-resolution mass spectrometry (UHPLC-HRMS)

Benzodiazepines and benzodiazepine-like drugs are the most common substances associated with drug-facilitated sexual assaults (DFSA); however, barbiturates are also detected occasionally. Segmental hair analysis provides useful information on the historic pattern of drug use, enabling differentiation between single exposure in DFSA cases and chronic use. However, sensitive and specific methods for barbiturate analysis in hair samples are needed. Herein, we present an ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) method for qualitative and quantitative determination of seven barbiturates in hair samples. Firstly, a hair strand was decontaminated and then freeze-milled in liquid nitrogen. Next, 50 mg of powdered hair was extracted with methanol in an ultrasonic bath for 10 min in the presence of 10 mg phenobarbital-d5. The supernatant was dried under nitrogen gas, and the pellet was dissolved in 100 mL mobile phase. Afterwards, 10 mL of the suspension was injected into the UHPLC-HRMS system. The present method involved two UHPLC conditions for determination of barbiturates (I) and identification of the structural isomers amobarbital and pentobarbital (II). This method showed satisfactory linearity in a range of 0.02–20.00 ng/mg for UHPLC conditions I and II, both with a high determination coefficient (0.9991–0.9999). Thus, this method was successful in determining barbiturate concentration in human hair after a single-dose exposure, showing its potential for application in the investigation of DFSA cases [38].

Barbiturates which are old pharmaceutical drugs are still widely used in medical treatment of epilepsy and for general anesthesia. To date, more than 2500 different barbituric acid analogs have been synthesized, and 50 of them were introduced into medical use over the last century. Due to their highly addictive properties, pharmaceuticals containing barbiturates are under strict control in many countries. However, by considering the worldwide problem with new psychoactive substances (NPS) the introduction of new designer barbiturate analogs into the dark market might serve a serious public health problem in the near future. For this reason, there is an increasing need for application methods for barbiturates monitoring in biological samples. The UHPLC-QqQMS/MS method for determination of 15 barbiturates, phenytoin, methyprylon and glutethimide was developed and fully validated. The biological sample volume was reduced to only 50 μL. A simple LLE (pH 3 with ethyl acetate) was successfully applied. The lower LOQ was 10 ng/mL. The method enables differentiation of structural isomers: hexobarbital and cyclobarbital; as well as amobarbital and pentobarbital. Chromatographic separation was achieved with the use of the alkaline mobile phase (pH 9) and Acuity UPLC BEH C18 column. Furthermore, the novel fragmentation mechanism of barbiturates was proposed, which may have a great impact in identification of novel barbiturates analogs introduced to illegal marketplaces. The presented technique has a great potential to be applied in forensic, clinical and veterinary toxicological laboratories, as was evidenced by the positive results of international proficiency tests [39].

2.6. Capillary electrophoresis

SPE-CZE has different methods, such as online, offline, and Inline. Inline SPE has many benefits such as less organic solvent consumption, less sorbent, and easy elution. The presence of these in different biological samples may alter because one person's metabolism varies with another. The detection of the sample was carried out using UV diode array detection at 214 nm. Ethyl Acetate/hexane of about 500µl was used for pre-treatment of the sample. For acidic, basic, and neutral compounds, HLB polymeric sorbent is used. 20 Na2B4O7 at pH 9.2, 150 mM Tris buffer at pH 7.8, and 50 mM ammonium acetate at pH 9.7 are used as Background electrolytes—the retention time of the analyte altered with a change in pH. At pH, less than 7 peak areas are more. The retention time of secobarbital and barbital was not much influenced at pH less than 7 [40].

Capillary electrophoresis is a simple instrument that can analyze a small number of samples in a short time. It is a highly effective technique. Many barbiturates exist in the form of enantiomers or racemic mixtures. These R and S forms can be separated using chiral capillary electrophoresis separation. Cyclodextrins are most widely used for the separation of such a mixture in CE. The compounds are divided based on the differences in the formation of complexes, their stability ratio. The migration time also increases With the mass-to-charge ratio. The changes in migration time also depend on the complex formed by cyclodextrin [41].

The drugs used during pregnancy may reach the foetus through the blood vessels which are connected. There are high chances for barbiturates to enter the foetus. For their analysis, the stool obtained after birth which is called meconium is collected. To eliminate other components which may block the capillary they are subjected to extraction techniques using cartridges. The polymeric cartridges have a high extraction efficiency compared to normal cartridges using a

suitable buffer. After extraction, they are subjected to capillary electrophoresis which can determine the drugs present in the stool [42].

The biological samples subjected to the surfactant result in the formation of micelles depending upon the concentration of surfactant added to the analyte. These micelles result in the movement of the different analytes with different velocities within the capillary. The process of acquiring kinetic movement of the analyte due to the surfactant within the capillary is called Micellar electro kinetic capillary chromatography. Drugs like butalbital, pentobarbital can be separated based on migration differences [43].

Table 3 Report of Capillary electrophoresis methods for barbiturates

Drug	Method	Matrix	Extraction	References
Secobarbital Phenobarbital Barbital	CZE-UV	Urine	Inline solid-phase extraction	40
Barbital Secobarbital	CZE-UV	Urine	Liquid-liquid extraction	41
Phenobarbital, Pentobarbital, Hexobarbital, Secobarbital.	CZE-UV	Meconium	Solid-phase extraction	42

2.7. Gas chromatography

The drugs which have a closer retention time can be both quantified and identified with GC. The main use of GC is to detect drugs with higher sensitivity. The efficiency of separation depends on the column. In GC, the columns are of different diameters, lengths but mostly glass and stainless steel. 3 or more columns are used for better results. Different stationary phases require different operating temperatures. If there is a difference in operating temperature, the resolution of components in GC decreases. Different drugs have different retention times which can be analyzed by changing the temperature of the column. The retention time alters with the flow rate of gas or change in temperature [44].

For analysis of weak acids like barbiturates from biological samples like urine, blood, or plasma. The extraction process can be done by adding organic solvents like ether. Then shake the contents and remove the aqueous layer. Filter and evaporate. The barbiturates give better resolution at 190-210 degrees. The barbiturates which can exhibit as enantiomers can be analyzed using the chiral stationary phase. GC and HPLC can detect barbiturates even at very low concentrations at accurate levels. Different alkyl-derived barbiturates, like 1,5-Dimethyl-5-ethyl barbituric acid,5-methyl-1-propyl barbituric acid, methyl-pentobarbital areas can be analyzed using the has an advanced chiral stationary phase in GC [45,46].

Ultra-fast GC columns reach up to 1200 degrees Celsius per minute. This can analyzed barbiturates within a short run time. In emergency conditions where a large number of samples are to be analyzed in such situations, the ultra-fast GC column contains high-speed GC that can be analyzed and separated more rapidly in less time [47]. Using GC-MS derivatization of barbiturates to make the separation, elucidation of their structure can be done. The underivatized compounds may not be suitable for given chromatographic conditions. Hence derivatization by various mechanisms like alkylation, changing into diastereomers, alkylation methods are followed. The reasons for derivatization are to make drugs stable during the analytical procedure, to give better mass spectra, and for separation purposes. Barbiturates generally get adsorbed due to the destruction of some amount of sample and due to attachment of these compounds to the surface of the column during their separation, which results in contamination of the column. When N, N- dimethylene is added to the barbiturates, the methylation process takes place. Due to the methylation of these compounds, these derivative barbiturates had better reproducibility, peak shapes and stability can be improved [48].

Table 4 Report of GC analytical conditions for Barbiturates

Compounds	Stationary phase	Carrier gas	Temperature	References
CSP in GC Enantiomer separation of N-alkylated barbiturates	Chiral stationary phase chiral-β-cyclodextrin	Hydrogen (40 KPa column head pressure)	Injector temperature, 250 °C; FID	45
GC/MS Metharbital Allobarbital Amobarbital Pentobarbital, Secobarbital,	Methyl silicone fused silica	Helium (15 mL/min)	Column temperature: 60 °C → 8 °C/min → 250 °C; injection temperature: 250 °C	46
Ultra-fast GC Amobarbital, Secobarbital Hexobarbital, Phenobarbital	Fused silica	Helium (1.0mL/min)	Injector temperature: 300 °C Oven temperature-200-280°C FID temperature: 340°C	47

2.8. High-performance thin-layer chromatography

As many of these above methods are expensive, in HPTLC along with densitometry analysis the solvent consumption is less. So, it can prevent pollution of the environment due to hazardous chemicals. The stationary phase used is a glass plate with silica gel Si $60F_{254.}$ And the mobile phase, dichloromethane–ethyl acetate–formic acid is used, and recovery of phenobarbital was reported to be 101.2% The LOD was reported as 0.4 g/spot [49].

2.9. Thin-Layer chromatography

Thin-layer chromatography is a simple technique and less costly compared with other methods. Lipophilicity of barbiturates is determined by the affinity e of the compound towards aqueous and organic phases. The period of retention time for various barbiturates towards the stationary phase in TLC was determined. They are up to 12 cm and later kept under UV light for detection of spots at 254 nm .5, 5-disubstituted barbituric acid derivatives. Such as Barbital Pentobarbital, Butabarbital, Amobarbital, 5-ethyl-5-(4, 4-diethyl hexyl), Phenobarbital, Aprobarbital, Butalbital. These are determined using 2 mobile phase methanol: water and methanol: buffer. 5-ethyl-5-(4, 4-dimethyl hexyl) was reported to have higher lipophilicity and barbital was found to have lower lipophilicity when compared to other compounds in both these mobile phases [50].

The properties of barbiturates based upon their structure with cyclodextrins can be identified using thin-layer chromatography. The cyclodextrins have the property to form cavity and inclusion host-guest complexes that will help in improving the stability of various barbiturates. The solubility of barbiturates in water can be increased by alkyl substitution. These large inclusion complexes will have more separation rate in TLC when compared to small guest complex molecules. The isomers of barbiturates that are substituted at the para position are found to have more retention-factor when compared to ortho and Meta position isomers. The β and γ cyclodextrin forms higher complexes and is more stable compared to α cyclodextrins. If the R_1 position is substituted with longer aliphatic groups, then the stability of barbiturates will be increased. The stability will decrease by cyclic substituents in α cyclodextrins but stability increases in β cyclodextrins. The stability is more for complexes formed by β cyclodextrin with thiobarbiturates having higher hydrophobicity [51,52].

Table 5 Report of TLC analytical conditions for Barbiturates

Compounds	Stationary phase	Mobile phase	Spraying agents	Reference
Phenobarbital	TLC plate with silica gel	n-hexane-acetone-methanol (8: 3: 0.5, v/v/v),	o-toluidine reagent	49

		n-hexane-acetone-butanol (12: 8: 0.5, v/v/v), and chloroform acetic acid (9: 1,v/v)		
Amobarbital, Phenobarbital Pentobarbital Talbutal,	Aluminium TLC plates	Methanol-water and methanol–Bates–Bower borate buffer solution	UV detection	50
Phenobarbital, Barbital, Butabarbital Allobarbital, Aprobarbital, Heptabarbital	Silica gel G coated plates	Chloroform: acetone ((9:1, v/v).	Mercuric Sulphate, diphenyl carbazone, Potassium Permanganate	51

2.10. Colorimetric methods

For colorimetric estimation of barbiturates, they form complexation with mercuric ions. The extraction of barbiturate using chloroform as an organic phase. The mercuric ions present in the complex which are not reacted are removed during the filtration process. The purple colour complex is formed when diphenyl carbazone is added to the complex. The chromogenic equivalence for quinobarbital is more about 115% and hexobarbital was 38% [53].

2.11. Spectroscopic methods

2.11.1. IR Spectroscopy

In IR each compound has a unique fingerprint region. Potassium Bromide is used for the comparison of various barbiturates. The barbiturates when subjected to grinding resulted in the change of their crystalline nature. The barbiturates have different polymorphs due to which resulted in complexity in studying the IR spectrum of barbiturates [54]. The barbiturate complex was prepared by reacting vanadyl (II) sulphate and barbituric acid. The presence of carbonyl groups resulting in the intense band due to vibrations of the carbonyl group was reported at 1766, 1720, and 1696 cm^{-1}. The intensity of the absorption band was more in the vanadyl (II) complex at the C_2 carbonyl group and was reported at 1760 cm^{-1}. The O-H stretching vibrations at 3390 cm^{-1}

2.11.2. UV Visible spectroscopy

There is no destruction of samples in UV visible spectroscopy. Thiobarbiturates are formed when the oxygen atom present in the carbonyl group is replaced with sulphur. The replacement resulted in the increase of its solubility due to which the hypnotic action also increased. When it was replaced with different derivatives they vary in their pharmacological action and duration of its action. The derivatives of thiobarbiturates can act as an antiviral and anticancer agent. The solvent used was 95%ethanol and 5% water. The λ max for thiobarbiturates was reported at 320 nm [55].

If there are 2 or more drugs that can absorb UV when kept UV along with barbiturates, they will interfere with barbiturates due to which their detection becomes difficult. UV method for simultaneous determination of barbiturates in serum. The extraction of the drug in serum using chloroform, 0.1M HCl, and sodium sulphate. Hydrolysis and partial differentiation were carried out. When there is a change in pH from 10 to 2 the absorbance doesn't change. But due to the occurrence of salicylates, the absorbance was found to be decreased for barbiturates. The barbiturate absorbance was measured at 239mμ. Because at 239mμ it can even detect the compounds which are interfering along with barbiturates. pH 10 alkaline solution was used in their determination because they reported the barbiturates which can act for a longer duration get affected due to the presence of stronger alkaline conditions [56].

Azo Dyes of barbiturates are formed when a coupling mechanism takes place between barbituric acid and diazotized nitro aniline compounds. The diazotization process occurs when aniline is reacted with sodium nitrite. The diazotized compounds are further reacted with barbituric acid and absorbance was measured by spectrophotometric methods. This results in the rich yellow colour of an azo compound, and it can be stored for up to 7 days. The temperature should

be between 0-5 °C during diazotization. The intensity of the colour produced depends upon the type of base used. The absorbance doesn't increase with the increase in the concentration of diazo nitro aniline to the barbituric acid. When surfactants are added they reported a very slight change in the change in absorption maxima. The absorption maxima are 418 nm for barbituric acid diazotized with ortho aniline, 380 nm for barbituric acid diazotized with meta-aniline, and 370 nm for barbituric acid diazotized with para- aniline reagents were reported [57,58].

2.12. Immunoassay methods.

The identification of secobarbital in hair samples using the Enzyme-linked immunoassay method. It is a sensitive technique for extraction of drugs from hair. Hair extraction buffers have been used. But the time taken for the analysis of drugs takes a longer duration. These drugs reach hair in different ways such as through passive diffusion or secretions [59]. Proper washing of hair samples is needed. As hair is an exposed area for various gases, pollutants, and chemicals. Organic and aqueous phase washing is done to remove any interfering substances. For washing dichloromethane, isopropyl alcohol is used. Incubation of sample with Hair extraction buffer and neutralization process. After incubation with enzyme conjugates, the antigen which was not bound to the drug was washed through several cycles. These are analyzed using spectrophotometric techniques.

In Radioimmunoassay methods, there is a chance for cross-reaction with the other drugs which have similarities in their structure. RIA are highly sensitive techniques for barbiturate analysis. XAD resins on TLC, immunoassay methods can be followed for analysis of urine samples. The false-negative and false-positive chances are more in EMIT and TLC techniques. Cross-reactivity of secobarbital with urine is more comparative to phenobarbital, pentobarbital, and amobarbital [60].

3. Conclusion

The above methods of analysis of various barbiturates in biological samples provide results of high sensitivity, selectivity, accuracy, and precision. There is a need for monitoring the patients who are under barbiturate therapy for determining the concentration of drugs in the body. Various degradation studies of barbiturates using HPLC to know the degraded products and amount of degradation undergone in different conditions. In forensic studies analysis extraction of samples and identification of barbiturates which are ingested by above analytical techniques. Derivatization procedures in some TLC and GC methods take a longer time for analysis. To improve the screening, the chromatographic methods are coupled with detection methods like Mass spectroscopy, UV Visible spectroscopic methods. There is a need for the development of various new methods for the easier extraction process, less time consumption, less cost, easier detection, and free from interfering compounds such as drugs taken by the patients along with barbiturates.

Compliance with ethical standards

Acknowledgments

All the authors have made equal contribution.

Disclosure of conflict of interest

All the authors declared that they have no conflicts of interest.

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