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Review Paper

Psychoactive Substances: Overview of their identification techniques

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Abstract

The rapidly increasing trend of the use of psychoactive substances among youth is alarming. The lack of awareness about their harmful health effects leads to their misuse. Hence, their manufacture, possession, and use are kept under control by the Government. Fast identification and quantification of these substances are of paramount importance. The methods like HPTLC and HPLC have been extensively used for their analysis and quantification. This review article covers the basics of psychoactive substances, their addictions, regulations, analysis, and identification with the help of HPTLC and HPLC.

Keywords: Psychoactive substance, HPLC, HPTLC, morphine, benzodiazapeine.

Introduction

A psychoactive drug, also known as psychotropic substance acts primarily on the central nervous system and thus affects function of the brain[1,2]. They are also therefore, termed as 'mind altering substances'. It has found its place in almost all human societies be it rural or urban. The use of these substances easily goes unnoticed leading to significant change in the perception of user and slowly user becomes psychologically dependent on these drugs[3-6]. Narcotic and psychotropic substances are chemicals that have an effect upon the body and mind. Psychotropic substances have various medical and scientific applications; however, their pleasurable effects often compel users to consume them regularly, leading to their misuse or abuse, despite the associated health risks and negative consequences[7-12]. Hence, their manufacture, possession and use are kept under control by Government[13-16]. Psychotropic substances may be natural, semi-synthetic and synthetic based on the sources of origin (Table-1). Natural psychotropic substances are mainly products of plant origin whereas synthetic psychotropic substances are manufactured in factories or clandestine laboratories and do not need any plant product as raw material. Semi-synthetic products are manufactured in factories or clandestine laboratories but they require plant product or substances that are derived from plant products as raw materials.

Psychoactive drugs can be classified into three categories on the basis of their pharmacological actions on the central nervous system (CNS)[17-21]; (i) stimulants: drugs that stimulate the CNS, cause elevation of mood and a sense of increased energy and strength (ii) hallucinogens: drugs which bring state of different feeling and perception that are not felt otherwise and (iii) depressants: drugs which depress the CNS, relieve tension and anxiety, produce a feeling of well-being, peace and contentment, and induce sleep (Table-2). These psychoactive substances are beneficial to the mankind but as per the usual human nature, these substances are equally misused for short term benefits.

Table-1: Classification and examples of psychotropic substances based on source.

Classification	Natural	Semi-synthetic	Synthetic
Example	Opium, Marijuans, Cannabis, Coca Leaves, Khat	Diacetyl Morphine or Heroin LSD	Ketamine Alprazolam

Table-2: Classification and examples of psychotropic substances based on effect on CNS.

Classification	Stimulants	Hallucinogens	Depressants
Example	Cocaine, Amphetamines, Caffeine and Nicotine	Lysergic acid diethylamide (LSD), Phencyclidine piperidine (PCP), Cannabis products such as Hashish	Alcohol, Barbiturate sedatives, Non-barbiturate sedatives (eg chloral derivatives, Benzodiazipineseg. Alprazolam), Opioid drugs (eg. morphine, heroin)

Psychoactive drug abuse and addiction

The misuse of drugs refers to the habitual consumption of a substance in quantities or via methods that are not sanctioned or monitored by healthcare professionals. Many drugs may be subject to abuse but this term 'drug abuse' is commonly used to denote the abuse of socially unacceptable mood-altering or psychoactive substances such as cannabis plant-products, lysergic acid diethylamide or LSD, opioids, cocaine etc.[22-24]. The inborn desire in human to get away from the hard realities of life occasionally drives them to resort to the abuse of psychoactive drugs that bring about profound pleasurable changes in his personality. The desired distraction obtained from these substances often drives the individual to repeat and continue their use leading to drug addiction. Drug addiction is a complex disease, and quitting is lot more difficult. Drug abuse and addiction have negative consequences for both individuals and society. Drug abuse hurts the people who take drugs and also the people around them. It hurts the body and brain of the addict, sometimes forever[25-31].

As per the report published by the World Drug Report 2018 [32], the number of new psychoactive substances reported annually has increased from 130 in 2009 to 503 in 2015 and 542 in 2019 world-wide (Figure-1)[32]. Each year some old substances disappears also. The illicit drugs have been used by about 275 million people in 2016 in which cannabis alone is used by about 192 million people and about 31 million people suffer from drug use disorders. The effect of injected illicit drugs by about 11 million people are depicted in Figure-2[33].

India has a vast heritage of traditional medicinal systems for various ailments[34-37]. Some of these medicinal formulations contain psychoactive substances like opium, marijuana, cannabis etc. Sometimes these formulations are abused or misused by the people to get temporary pleasure. People are unable to utilize the benefit of the traditional systems of medicine due to lack of knowledge and quality control measures.

As per the 2019 report on the magnitude of substance use in India by the Ministry of Social Justice and Empowerment, the most commonly misused substances in India are alcohol, cannabis, and opioids (Figure-3). Within the category of cannabis, both legal forms like bhang and illegal forms such as ganja and charas are included. Similarly, the opioid category encompasses opium, heroin, as well as impure forms like smack or brown sugar, along with various pharmaceutical opioids.

Psychoactive drug abuse regulations

The psychoactive substances are beneficial to the mankind but as per the usual human nature, these substances are equally misused for short term benefits. Due to this fact of misuse or abuse of these substances, they are kept under control by United Nations Drugs Control Program (UNDCP)[13-16]. In recent times, an alarming and unprecedented increase have been observed in new type of psychoactive substances and also existing drugs. This situation presents a challenge not only for law enforcement staff but also to the technical and scientific staffs of forensic laboratories to quantify their presence in the materials obtained.

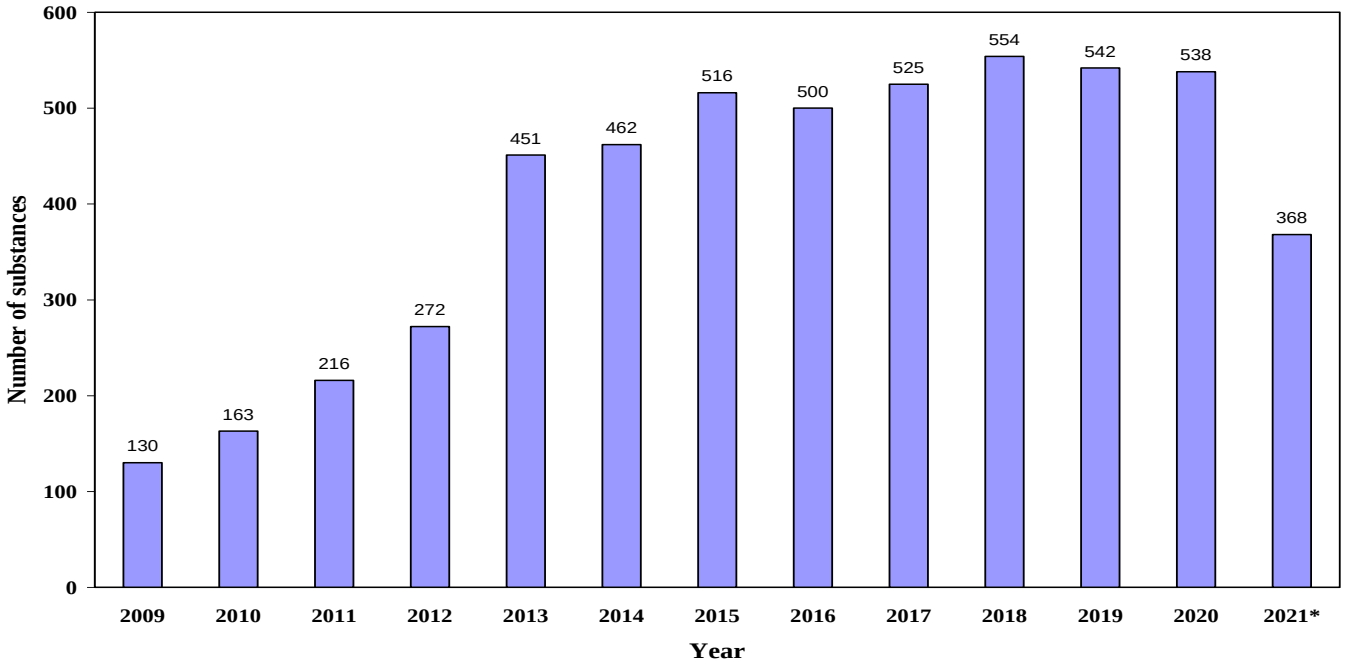

Figure-1: Number of new psychoactive substances reported annually. (*data collection for 2021 was not completed till the report was published and therefore subject to change).

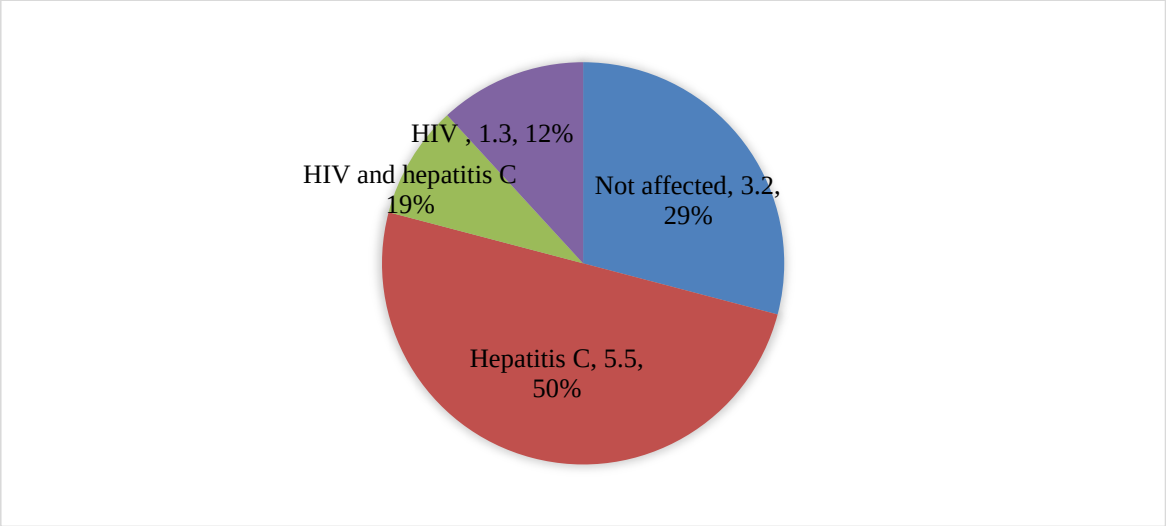

Figure-2: Impact of injected illicit drugs worldwide in 2016.

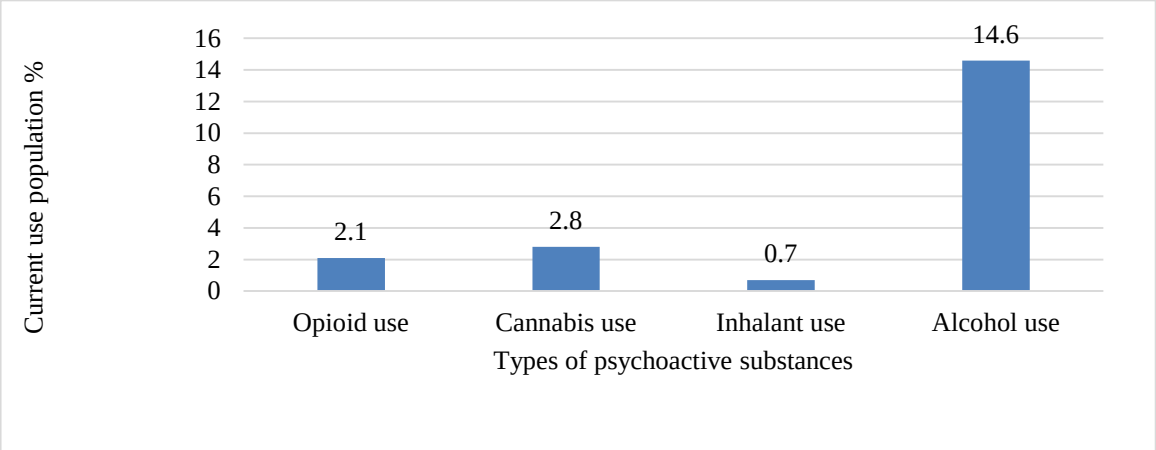

Figure 3: Use of psychoactive substances as per 2019 GoI report.

The diversion of illicit pharmaceuticals containing narcotic drugs and psychotropic substances for abusive purposes is a significant issue in India. To address this problem, the Indian government has implemented the Narcotic Drugs and Psychotropic Substances (NDPS) Act, 1985, which empowers law enforcement officers from both the Central and State governments to enforce it.

These agencies when ever get information about misuse of any preparation containing psychotropic substance then they seize such preparations and send them to designate forensic or revenue laboratories for detection and quantification of suspected psychotropic substance present in them for prosecution purposes. It is necessary to test each of the seized samples rapidly, precisely, and accurately since the test result serves as the foundation for the trial of the accused. The law imposes harsh penalties for drug trafficking and related offenses. The NDPS Act 1985 has been amended as per requirements by the GoI in 1988, 2001, and 2014.

Psychoactive drug analysis and quantification

The psychoactive substances are also useful medicine if used with proper medical prescription and used for the treatment of various ailments[38,39]. They are the part of modern medicine and hence, they do have their analytical standards in various National and International Pharmacopoeias. These psychoactive substances are also covered by Drugs & Cosmetics Act and Rules of various countries. Hence, either for use or abuse, their analysis and quantification always have the role to play.

Besides psychoactive substance of modern medicine, there are many commercially available Ayurvedic and Unani formulations which do contain psychoactive substances viz. opium and cannabis. Mention of such formulations is already in official books of Ayurveda and Unani System of Medicine (Ayurvedic Formulary of India and Unani Formulary of India) and in authorized classical books of Ayurveda & Unani (listed under First Schedule Drugs & Cosmetics Act, 1940) which contain opium and cannabis as one of the ingredients in

significant quantity along with other drugs of natural origin (herbal/animal/mineral). It is surprising to mention that till date, no method for analysis of such formulation has either been published in any pharmacopoeia, by National Authorities of AYUSH nor by any analyst/researcher in journals of repute.

Psychoactive substances are controlled substances. Without authorization of appropriate authority their formulations / material seized by law enforcement officers, nor reference material of used active psychoactive ingredients can be obtained/procured. Hence, analytical work on such psychoactive substances and their preparations cannot be done in any laboratory. Analytical/research work can only be done in laboratories authorized by National or State Government Authorities for the purpose. Advantage of being chemist of an organization which is the nodal and authorized laboratory for analysis of narcotic drugs and psychotropic substances under NDPS Act and rules there under has been taken to do analytical work on some psychoactive drugs and on their formulations used in modern medicine and indigenous system of medicine i.e. Ayurveda and Unani and have develop validated analytical method for analysis of psychoactive substance present in them using those techniques which are capable to separate psychoactive substance from other ingredients of formulations.

A number of research groups have used a range of analytical techniques for analysis and quantification of including psychoactive substances (Figure-4)[40-45].

Out of the different analytical techniques investigated, chromatographic techniqueslike High Performance Thin Layer Chromatography (HPTLC)[46-51] and High Performance Liquid Chromatography (HPLC)[52-56] have been widely used. Identification/ quantification of some of the psychoactive substances and suitable solvent system has been summarized in the Table-3 [57-92].

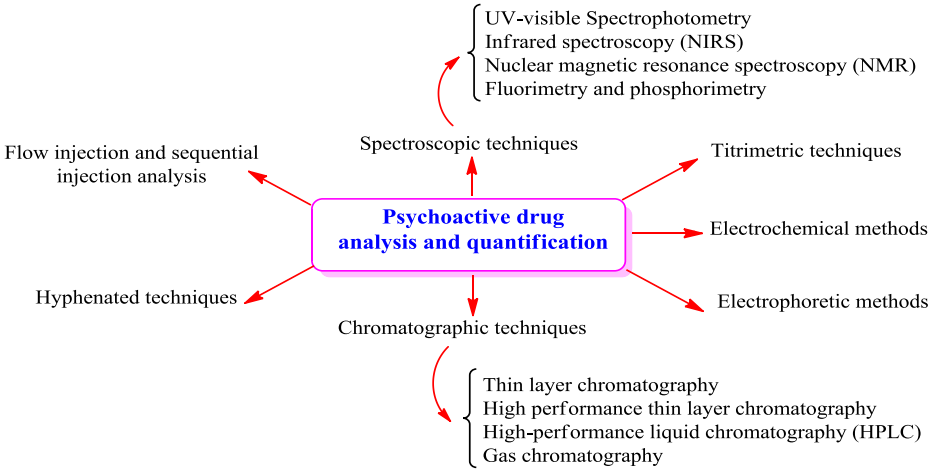

Figure-4: Analytical techniques for psychoactive drug analysis and quantification.

Table-3: Suitable solvent systems for some of the psychoactive substances.

PAS or Class of PAS/ analyte	Source/ matrix/ sample	Mobile phase	Technique	Ref.
Opiate analysis	Urine	Hexane-chloroform-diethylamine (5:3:0.7)	TLC	57
Opiate analysis	Urine	Chloroform-hexane-triethylamine (9:9:4)	TLC	57
Opiate analysis	Urine	Benzene-dioxane-ethanol-ammonia (5:4:0.5:0.5)	TLC	57
Morphine	Urine	Ethyl acetate: methanol: ammonia (8.5:1:0.5)	HPTLC	58
Morphine	Human plasma	Chloroform - isopropyl alcohol (9:1)	HPLC-UV	59
Morphine	Rat brain	Acetonitrile/0.1 M acetate buffer 4.5:5.5 (v/v %)	HPLC	60
Morphine	KVR	Ethyl acetate–methanol–ammonia solution (8.5:1:0.5)	HPTLC	61, 62
Morphine	KVR	*N*-heptane sulfonate sodium salt–acetonitrile (7:3)	RP-HPLC	62

Opiates and Cocaine	Urine	A) Ammonium hydrogen carbonate (B) Methanol, 5-90-5% B in A	RP-HPLC	63
Cocaine	Blood/ urine	(A) 5% Acetonitrile + 0.05% Formic acid (B) 100% Acetonitrile + 0.05% formic acid, 95-60% B in A	HPLC	64
Cocaine	Urine	Acetonitrile + Ammonium formate 5mM + Formic acid	HPLC	65
Cocaine	Serum	(A) 0.1% Formic acid + Ammonium formate 1mM (B) Acetonitrile + 0.1% Formic acid + Ammonium formate 1mM	HPLC	66
Cocaine	Plasma	(A) Acetonitrile (B) PO_4 3- buffer, 10-50-10% A in B	HPLC	67
Cocaine	Hair	(A) 0.01% Formic acid (B) Methanol (C) Acetonitrile 10%, B + 70-30-70% A + 20- 60-20% C	HPLC	68
Cocaine	Hair	Methanol:Acetonitrile:Potassiumdihydrogen phosphate buffer (10:15:75) + 0.25% Triethylamine	HPLC	69
Cocaine	Saliva	(A) Methanol + Ammonium formate 10 mM (B) H_2O + Ammonium formate 10 mM 6-41.2% A in B	HPLC	70
Benzodiazepines (alprazolam, bromazepam, clonazepam, clotiazepam, etizolam, flunitrazepam, lorazepam)	Human breast milk and plasma	Gradient elution with 10 mM ammonium acetate and acetonitrile	HPLC	71
Benzodiazepines	Saliva	Linear gradient (water/formic acid 5mM: acetonitrile/formic acid 5mM; v:v) from 98:2 to 0:100 in 5.0min, followed by isocratic elution at 100% B for 1.0min	HPLC	72-73
Benzodiazepines	Blood serum	Acetonitrile, methanol and ammonium acetate (0.05 M),	HPLC	74
Benzodiazepines	Urine		GC-MS	75-77
Benzodiazepines	Nails		GC-MS	78
Benzodiazepines	Hair		GC-MS	79
Sertraline Hydrochloride and Alprazolam	Pharmaceutical preparation	KH_2PO_4 buffer: acetonitrile 40:60 v/v	RP-HPLC	80
Alprazolam	Human serum	Acetonitrile: water, 95:5	NP-HPLC	81
Alprazolam (ALP) and propranolol hydrochloride (PNL)	Pharmaceutical preparation	Acetonitrile-25 mM ammonium acetate buffer and 0.2% triethylamine 35: 65 (v/v)	RP-HPLC	82
Alprazolam (ALP) and Propranolol hydrochloride (PNL)	Pharmaceutical preparation	Chloroform-methanol-ammonia 7: 0.8: 0.1	HPTLC	82
Alprazolam	Pharmaceutical preparation	0.02 M buffer solution of phosphates (pH 6.0) and acetonitrile (4.5:5.5)	RP-HPLC	83
Alprazolam (Alp) and Fluoxetine Hydrochloride (Flx)	Pharmaceutical preparation	Acetonitrile: Water (7.5:2.5)	RP-HPLC	84
Alprazolam (ALZ) and sertraline (SER)	Pharmaceutical preparation	Acetone/toluene/ammonia (6:3:1)	HPTLC	85
Alprazolam (ALZ) and sertraline (SER)	Pharmaceutical preparation	Acetonitrile and phosphate buffer (5:5)	RP-HPLC	85

Ketamine, xylazine, and midazolam	Canine plasma	Acetonitrile –methanol –10 mM sodium heptanesulfonate buffer (4.4:1:4.6)	HPLC	86
Ketamine, norketamine, and dehydronorketamine	Plasma	Acetonitrile:0.03 mol/L phosphate buffer (2.3:7.7)	HPLC	87
Paracetamol (PAR), theophylline (THE), amphetamine (AM), methamphetamine (MAM) and caffeine (CAF)		Phosphate buffer, acetonitrile and methanol in the ratio 85:10:5 v/v/v	HPLC	88
Mexiletine hydrochloride	Pharmaceutical preparation	Buffer – acetonitrile (60:42, v/v)	HPLC	89
Olanzapine	Pharmaceutical preparation	Methanol- ethyl acetate (8.0+2.0 v/v)	HPTLC	90
Stavudine (SV), lamivudine (LV) and nevirapine (NV)	Pharmaceutical preparation	Sodium phosphate buffer (containing 8 mM 1-octanesulphonicacid sodium salt) : acetonitrile (4:1 v/v)	RP-HPLC	91
Stavudine (SV), lamivudine (LV) and nevirapine (NV)	Pharmaceutical preparation	Chloroform:methanol (9;1, v/v)	HPTLC	91
Levocetirizine Dihydrochloride and Montelukast Sodium	Pharmaceutical preparation	Toluene: ethyl acetate: methanol: ammonia (2.5:7:2.5:1, v/v/v/v)	HPTLC	92
Levocetirizine Dihydrochloride and Montelukast Sodium	Pharmaceutical preparation	Sodium dihydrogen phosphate buffer (0.02 M): Methanol (25:75, v/v)	RP-HPLC	92

High Performance Thin Layer Chromatography (HPTLC)

This is an advanced version of TLC[46-51]. This also comprises of integrated software controlled sophisticated instruments. It is a powerful analytical method incorporating standardized methodology as well as validated methods equally suitable for both qualitative and quantitative investigative tasks.

HPTLC is advantageous for many reasons such as: i. Visual chromatogram, ii. Simple sample preparation, iii. faster and economical analysis as number of samples can be analysed simultaneously, iv. Enables the complicated separation of samples of divergent nature, v. No contamination or interference from previous analysis, vi. Detection limit in nanogram range. vii. Large no of theoretical plates in minimum area of plates, viii. Low maintenance cost.

HPTLC-based methods are a viable alternative for routine analysis due to their numerous advantages. Method development and validation are interdependent processes, as operational parameters are only considered acceptable if performance requirements are met. Consequently, validation is a critical step in determining the method's reliability and reproducibility, as outlined in references[93-94]. The International Conference on Harmonization (ICH) guidelines are instrumental in defining the validation parameters[95]. The various steps involved in HPTLC are:

Sample preparation: A suitable solvent should be there to dissolve the sample to be analysed without dissolving the impurities present. The resulting solution will be directly applied on HPTLC plate[96].

Selection of stationary phase: In most of the reported studies, the development of HPTLC method has been done with Silica Gel 60 F_{254} on either glass or aluminium backing[97-98].

Layer pre-washing: Plates should be prewashed to improve the reproducibility and robustness of the analysis[95,96].

Selection and optimization of mobile phase: Literature survey will help in selecting the mobile phase for HPTLC method along with traditional-trial and error method[99].

Sample application: There are two ways to transfer sample on the HPTLC plate that are commonly followed: contact application and spray techniques. This will determine the overall quality of the separation[97, 99].

Chromatogram development: The chambers such as horizontal-development, twin-trough, and flat-bottom help in the development of HPTLC plates.

Plate labeling: The plates should be labelled for identification.

Derivatization: It is one of the advantages of HPTLC that the sample fractions after chromatography do not decompose however, remains stored on the plate that can be derivatized later if required. The derivatization sometime become necessary for those samples that do not respond to visible or UV light, but after derivatization, that can be detected. In many cases, drugs/ samples can be identified by specific reagents[96].

Documentation: A digital documentation is associated with each developed plate which is documented with suitable software. The obtained results have been saved and stored.

Detection: Various zones can be detected by their colour, fluorescence, quenching of fluorescence or UV-absorbing with a reagent[96].

Quantitation: Generally quantitative evaluation is performed with the scanner.

Validation of developed method: According to the ICH guidelines - validation of analytical procedures - text and methodology Q2 (R1), the following validation parameters are typically monitored for HPTLC method: (a) specificity; (b) linearity; (c) precision; (d) limit of detection and quantitation; (e) robustness; (f) accuracy[95].

High Performance Liquid Chromatography (HPLC)

HPLC is a powerful analytical tool to separate, identify and quantitate the compound along with the related impurities present[100-103]. With the recent advancements in the technique, very low concentrations of compounds can easily be identified. HPLC has a wide range of applicability for analysis with very small amount of samples ranging from pharmaceutical samples to forensic and industrial samples.

The working principle of HPLC is that a well dissolved sample solution is injected into a stationary phase (column) and mobile phase is pumped at high pressure which facilitate the separation of sample[104,105]. This is based on the chemical structure and nature of the analyte.

The packing material and sample to be analysed show some interaction and this interaction decides the retention time. Based on difference in retention time, different constituents of a sample are eluted at different intervals. Thereby, the separation of the sample ingredients is achieved.

The important requirements for a HPLC method development are; understanding of chemical properties of the sample, preparation of sample solution, optimization of chromatographic conditions, standardization, and validation[100-103]. The validation parameters are similar to HPTLC as per ICH guidelines[95].

Conclusion

A psychoactive drug acts primarily on the central nervous system and thus affects function of the brain. These substances have several medical and scientific applications. But their regular use leads to addiction. The rapidly increasing trend of the use of psychoactive substances among youth is alarming due to the lack of awareness about their harmful health effects. The Government have kept control over their manufacturing, possession, and use to avoid their misuse. Many times, large amount of these substances is found in illegal possession which requires fast identification and quantification. Various researchers have worked in this direction using the methods like HPTLC and HPLC. Which of these two methods is preferred for the analysis of the substance depends on several factors such as nature of substance and specific analysis requirement.

This review article covered the basics of psychoactive substances, their addictions, regulations, analysis, and identification with the help of HPTLC and HPLC. HPLC helps in separating a wide range of complex mixtures and provides good selectivity and sensitivity even if the concentration of analyte is very low. The bottleneck of HPLC is it's high operating cost and sophisticated instrumentation. HPTLC, on the other hand, is more user-friendly technique that allows rapid and cost-effective analysis of multiple samples and that too without any expensive instrumentation. The drawback of using HPTLC is lower resolution as compared to HPLC. So HPTLC is a better option for fast and cost-effective qualitative analysis.

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