Determination of alkaloids for Qualitative and Quantitative analysis by HPLC: A Review

[1]Manisha P. Bisen, [2]Ujwala D.Larokar, [3]Sandhya M. Bagde, [4]Dr.Ujwala mahajan
[1,2,3,4]Dadasaheb Balpande Collage of pharmacy, Besa Nagpur

Abstract

Alkaloids are biologically active compounds widely used as pharmaceuticals and synthesised as secondary metabolites in plants. Many of these compounds are strongly toxic. Alkaloids are a group of naturally occurring chemical compounds that contains mostly basic nitrogen atoms. Alkaloids are produced by a large variety of organisms which includes bacteria, fungi, plants and animals. In this review identify the retention time of various alkaloids by using HPLC Method. For atropine Pentafluorophenyl C18 column with Mobile phase is Water, acetonitrile acidify with 0.08% of trifluoroacetic acid are used and Retention time of Atropine is 7.7 minutes. For ajmaline column- RP18e column (100 × 4.6mm) with Mobile phase is 0.01M(PH3.5) phosphate buffer containing 0.5%glacetic acid &acetonitrile and Retention time of Ajmaline is 6.05minutes.column- C18(250 mm4.6 mm , 5um)with Mobile phase is Acetonitrile : methanol : water (32:48:20v/v)PH5.2 with phosphoric acid . For the above study conclude that many of alkaloids are analyse qualitatively & quantitatively using HPLC system.The present study showed that there are number of HPLC analytical methods are available for the qualitative and quantitative analysis of alkaloids.

KEYWORDS: Alkaloids, Atropa Belladonna, HPLC, Mobile phase ,Retention time, quantitative analysis, qualitative analysis.

INTRODUCTION

Alkaloids are a group of naturally occurring chemical compounds that contains mostly basic nitrogen atoms.[1] Alkaloids are produced by a large variety of organisms which includes bacteria, fungi, plants and animals.[2]Alkaloids are produced by a large variety of organisms which includes bacteria, fungi, plants and animals.Many alkaloids have been used for several hundreds of years in medicine and even today it's a still prominent drug.[3]In most of the human history, alkaloids from plant extracts have been used as ingredients in liquidmedicinal and poison.[4] High-performance liquid chromatography (or High pressure liquid chromatography, HPLC) is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds.[5] HPLC analysis of alkaloids is performed by means of ion-exchange, reversed-phase, ion-pair, and

straight-phase chromatography .HPLC has found limited applications in the analysis of alkaloids.[6] The stationary phases employed in HPLC are usually chemically bonded ion-exchange groups (alkyl sulfonic groups) on silica gel HPLC mainly utilizes a column that holds packing material (stationary phase) , a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules.[7]

Aropine

Atropa belladonna, commonly known as belladonna or deadly nightshade. The plant Atropa belladonna belongs to the family Solanaceae is a perennial herb.The plant contains tropane alkaloids , including atropine, scopolamine, and hyoscyamine, which are used as medicinal, herbal and homeopathic remedies**.**It is commonly known as belladonna, deadly nightshade, devils herb, divale, dwale, devils cherries, gray morel, naughty mans cherries, and poison black cherry. [8] In the year 2017 studied Quantification of Atropa belladonna development validation method by HPLC. The leaves of Atropa belladonna are characterized by the presence of the alkaloid Atropine, known for antimuscarinic activity .Pentafluorophenyl C18 column with Mobile phase is Water, acetonitrile acidify with 0.08% of trifluoroacetic acidRetention time of Atropine is 7.7 minutes.[9]

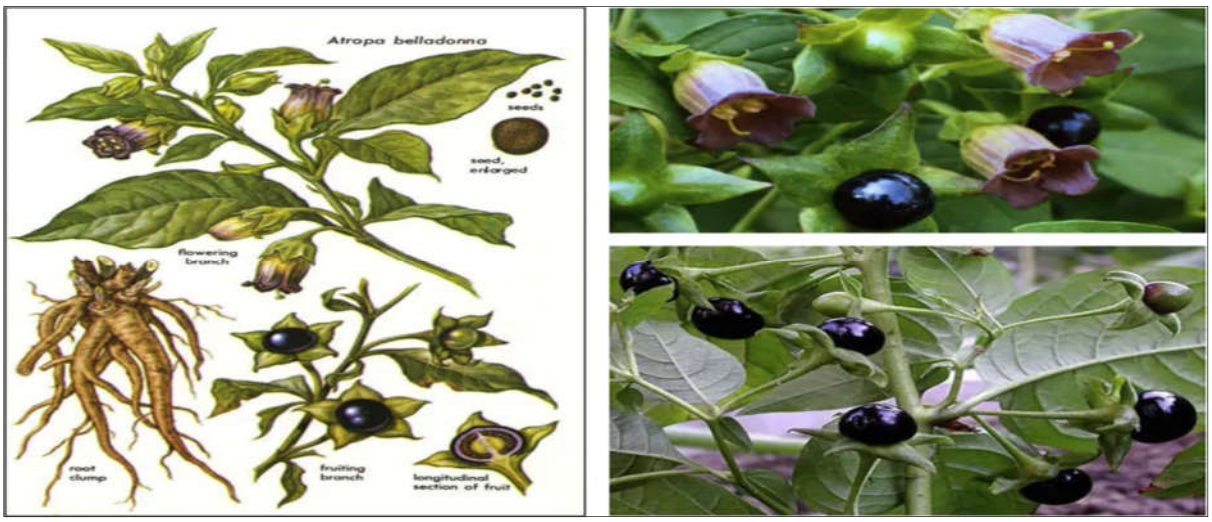

Figure 2: The features of the Atropa belladonna plant showing the leaves, roots, and berries Atropa belladonna - Deadly Nightshade

Reserpine

Rauvolfia (Rauwolfia serpentina (L.) Benth. ex kurz; family: Apocynaceae) is a small woody perennial from India and the East Indies. Reserpine, the major alkaloid of the root, was the first major tranquilizer to be used, especially for the treatment of paranoia and schizophrenia. It was also used as a substance that lowers blood pressure and controls hypertension. Interestingly, its roots were long used in India for treating mental illness and snakebite, known to medicine men and peasants as the “Insanity herb” or “snakeroot”[10] A sensitive and reproducible reversed-phase high-performanceliquid chromatography (HPLC) method using photodiode array detection is established for the simultaneous

quantitation of important root alkaloids of Rauvolfia serpentina, namely,reserpine, ajmaline, and ajmalicine[11] Quantitative determination of reserpine, ajmaline, ajmalicine in Rauwolfia serpentina by reversed phase by HPLC.The roots of Rauwolfia serpentina are characterized by the presence of the alkaloid Ajmaline, known for Antihypertensive agent. column- RP18e column (100 × 4.6mm) with Mobile phase is 0.01M(PH3.5) phosphate buffer containing 0.5%glacetic acid &acetonitrile and Retention time of Ajmaline is 6.05minutes.The limits of detection are 6, 4, and 8 µg/mL forajmaline, ajmalicine, and reserpine, respectively, and the limits of quantitation are 19, 12, and 23 µg/mL for ajmaline,ajmalicine, and reserpine, respectively. The developed method is simple, reproducible, and easy to operate. It is useful for the evaluation of R. serpentina[12]

Figure 2: The features of the Rauwolfia

Vincristine

Catharanthus roseus (L.) G. Don (Family Apocyanaceae),known in trade as Vinca, is a pantropical species occurringchiefly in the West Indies and Madagascar and is extensively cul-tivated in many states of India. The plant is known to producemore than 200 important compounds (mainly alkaloids). Vincaalkaloids, mainly vincristine and vinblastine.catharanthus roseus L. is a potent medicinal plant belonging to Apocynaceae family. In a number of countries, different parts of it are traditionally used in the treatment of various diseases, e.g. diabetes, menstrual irregulations, hypertension, cancer, etc.[13] A simple reversed-phase liquid chromatographic method is developed for the simultaneous quantitation of the anticancerous drugs vincristine, vinblastine, and their precursors catharanthine and vindoline using a Merck Chromolith Performance reversed-phase high-performance liquid chromatography column. A better resolution is obtained in comparison with available particulate-type C18 columns. The column provides good reproducibility and peak symmetry. Chromatography is carried isocratically with a mobile phase of acetonitrile-0.1M phosphate buffer containing 0.5% glacial acetic acid (21:79, v/v; pH 3.5) at a flow rate of 1.2 mL/min and UV detection at 254 nm. Parameters such as linearity, limits of quantitation (LOQ) and detection (LOD), precision, accuracy, recovery, and robustness are studied. The method is selective and linear for alkaloid concentration in the range 0.25 microg-25 microg/mL. The LOQ and LOD are 25, 46, 56, and 32 microg/mL and 8, 14, 18, and 10 microg/mL, respectively. The results of accuracy studies are good. Values for coefficient of variation are 2.50, 1.82, 1.33, and 1.13, respectively. The percent recovery of the alkaloids was found to be 96%, 97%, 98%, and 98%, respectively. Peak purity and homogeneity of these compounds in plant extract is studied using a photodiode-array detector. This simple and rapid method of analysis is

applied for the determination of these alkaloids in a large number of leaf extracts of Catharanthus roseus.similarly M.M. Gupta1, et al.in the year 2005studied Simultaneous Determination Vincristine, Vinblastine, Catharanthine, and Vindoline in Leaves of Catharanthus roseus by High-Performance Liquid Chromatography.Theleaves,arial part of Catharanthus roseus are characterized by the presence of the alkaloid, vinblastine use for Anticancer agent. HPLC column - C18microsorb –mv column (250mm× 4.6mm) with Mobile phase is Methanol ,phosphate buffer PH6.0,acetonitrile used. Retention time of vinblastine is 6.80 minutes [14]

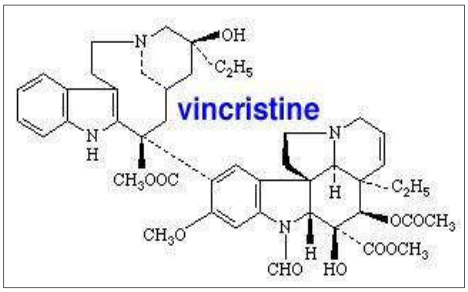

Figure 3: Structure of vincristine

Harmine

Pegan genus Peganum harmala L. belongs to the Zygophyllaceae (the Caltrop plant family) consists of 30 genera and 230 species. Are grows in the tropic, subtropics and warm regions (6,7). This family contains rich Lycorine, Haeman-thamine, Galanthamine and Elaeagine alkaloids especially harman and harmine which is normally found in the (Peganum harmala L.) Qualitative and quantitative analysis of alkaloid component in seeds from Peganum harmala L. extracts Abstract: Peganum harmala L. represents the major rich plant of alkaloids harmine and harmanline. In this study, one of these alkaloids was detected qualitatively and quantitatively using different extraction methods: aqueous, methanol, and alkaloid. In comparison between standard harmine and the three extracted using TLC technique, different spots were appeared from the three extraction methods and the color looked like the standard. While when run the extracts on HPLC in comparison to standard harmine, three peaks were observed and retention time of each extract was recorded to calculate the concentration of alkaloid. The best extraction method was the alkaloid extraction that give two peaks quite similar to that in the standard and almost the same retention time. This study shows that there are many important compounds in the alkaloid extraction of P. harmala of Iraqi species.[15]after that in the year 2013studied Qualitative & quantitative analysis by HPLC of major Peganum harmala alkaloids of different stages of development.The Rhizome of Peganum harmala are characterized by the presence of the alkaloid, Harmol use for Strong Disinfectant.HPLC column- C18 ODS type (150mm*4.6mm 3 micron) With Mobile phase is0.5%formic acid .17%water in methanol & buffered with triethylamine and Retention time of harmol is 2.21minutes[16]

Quinine

Cinchona has been known in Europe since the 1640s and has been used in treating malaria since the 1820s The extraction, isolation, and purification of quinine and cinchonine .The genus Cinchona belongs to the Rubiaceae family.Cinchona alkaloids are composed of 4 main alkaloids (quinoline alkaloids), namely quinine (QN), quinidine (QD), cinchonine (CN), and cinchonidine.Cinchona alkaloids have pharmacological activities as antimalarial , anticancer , antioxidant , anti-diabetic, antifungal , muscle anti-cramp, hair growth stimulant ,antimicrobial , antiobesity , antiplatelet , antivirus , anesthetic and antipyretic.Quinine and current synthetic antimalarials may suffer competition from other drugs or by the development of a vaccine.Quinine is used as a bitter flavouring in drinks and in many foodstuffs.quinine- and cryptolepine-based antimalarials serve as valuable alternatives to artemisinin-based combination therapies. Cinchona alkaloid quinine has also been used for a very long time, andit continues to serve its purpose in the management ofmalaria. It is used for uncomplicated malaria in the firsttrimester of pregnancy and, in some cases, in the second andthird trimesters. It is also used in severe malaria and intreatment failures associated with the ACTs.[17]Quinidine is known as antiarrhythmic, anti-depressant, epilepsy therapy drugs, and used in dementia treatment.After that J. KARBWANG et al.in the year 1989 studied Determination of quinine &quinidine alkaloids in biological fluid by HPLC. Determination of quinine & quinidine alkaloids in biological fluid by HPLC.The bark part of Chinchona calisaya are characterized by the presence of the alkaloid quinine use for antimalarial agent. HPLC column - INETEX-XB-C18(150mm× 2.1 mm)2.6um with Mobile phase is 0.2Mammonium formate buffer with PH 3.0 & water (10:90) and Retention time of quinine is 11 minutes[18]

Qualitative And Quantitative Analysis Of Alkaloids

Sr. no	Name of Alkaloids	Plant name	Part of plant use	Mobile phase	column	Retention time	Quantification value
1	Atropine	Atropa belladonna	leaves	Water, acetonitrate, acidify with 0.08% of trifluroacetic acid	Pentaflur ophenyl C18	7.7min	3.75µg/ml
2	Reserpine	Rauwolfia serpentina	root	0.01m (3.5ph) phosphate buffer containing	RP-18e column (100	6.05min	19µg/ml

				0.5% glacial acetic acid & acetonitrile	×4.6mm)		
3	Vincristine	Catharanthus roseus	Leaves & stem	Methanol : acetonitrile : amoniumacetate buffer with 0.1 tri ethyl amine	C18 250 mm % 4.6mm 5mm	21.5min	5.0µg/ml
4	Harmine	Peganum harmala	rhizomes	0.5 formic acid 17% water in methanol buffered with tri ethylamine	C18 ODS type (150mm ×4.6mm 3mm)	2.2min	21.60µg/ml
5	Quinine	Chinchona calisaya	Bark & root	0.2M Amonium formate buffer with pH3 and water (10:90)	KINETE X- XB C18 (150×2.1 mm 2.6mm)	11 min	6.8µg/ml

Conclusion:

By complile this study helpful for future or further study. For the above study conclude that many of alkaloids are analyse qualitatively & quantitatively by using HPLC system. This data will be helpful for researcher who make carry the analysis of alkaloids on HPLC system. The present study showed that there are number of HPLC analytical methods are available for the quantitative & qualitative analysis of alkaloids .The future scope of this review to summerise the total analysis of alkaloids by HPLC method .By referring this review research , are further developed the new HPLC analytical methods which are more convenient and rapid qualitative and quantitative analysis of alkaloids.

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