

High Pressure Liquid Chromatography Used qualitative Analysis - Vinca

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ABSTRACT

High pressure liquid chromatography technique used in a alkaloids were **VINCA** plant components are separated from the technique

Keywords: High pressure liquid chromatography, vinca plant in flowers,leaves, vinca fruits, dectors, application.

I. INTRODUCTION

Alkaloids are a important of plant metabolites, they are basically nitrogen containing



compounds were there,there are cultivated in a Africa, Australia, Eastern Europe, India ,Tivan, Thailand.andSouth Florida.

Common name :Periwinkle

Biological name: Catharanthusroseus,

Family: Apocynaceae,

Chemical class:

Vincais belongs to dimer indole group of alkaloids,Two indole groups are attached with **C-C linkage**.

Vinca parts

It vinca plants are two parts were there ,first is a majorvinca and another one is a minor vinca, **Major vinca** is **large** leaflets and **large flower** than **Minor vinca**.

Morphology:

Type: The plant is an annular or perennial.

Size: 0.5 to 1meter in height

Leaves: Generally ovate,oblong

Flowers: 2 to 3 in cymes, axillary,

Fruit: A follicle, cylindrical.

Taste: Bitter,

Odour: Slight.

Microscopy

The **Transverse section** of vinca leaf shows the presence of following parts

- ❖ Upper epidermis
- ❖ Mesophyll
- ❖ Lower Epidermis
- ❖ Midrib

Chemical constituents

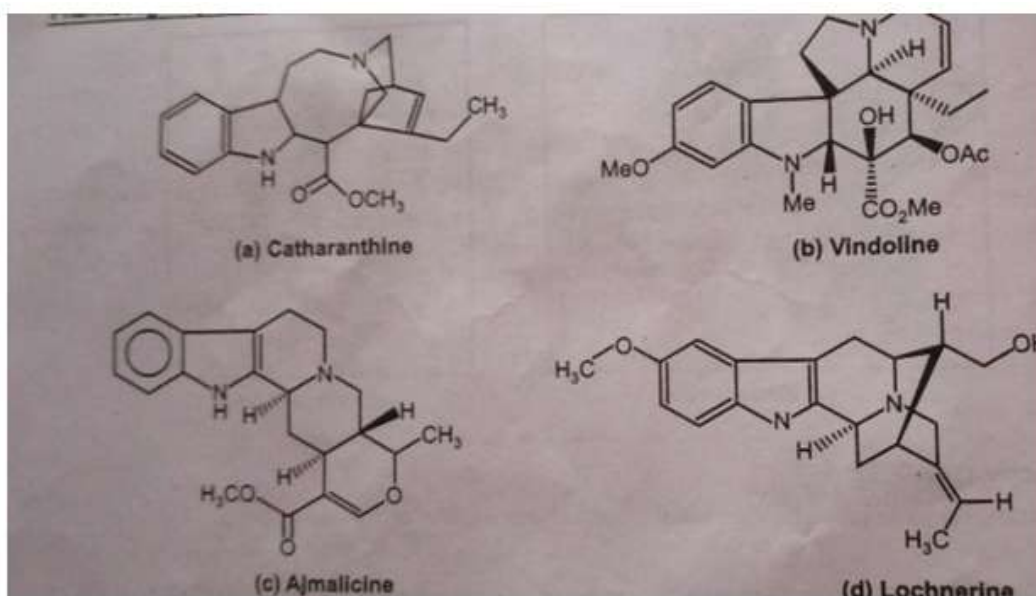
Vinca contains mainly indole and Tetrahydroalstonine.

Indole and indoline alkaloids

- Ajmalicine
- Lochnerine
- Serpentine

➤ Catharanthine

➤ Vindoline



Tetrahydroalstonine

It is a dimeric indole bases of monoterpene type, these are two types. Vincristine (VIC), vinblastin(VIB).

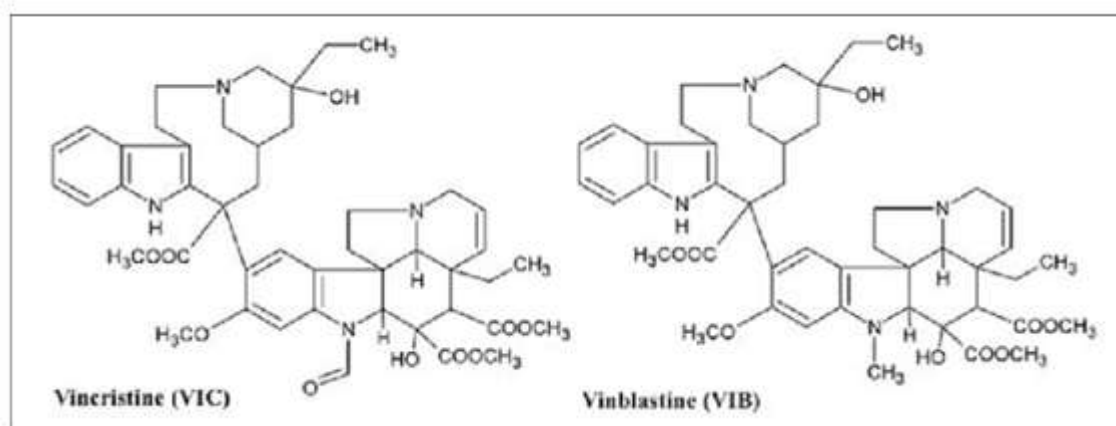
General method of extraction

- Svoboda's method
- Gradient PH extraction

Quantitative analysis

In this technique in HPLC method are used Chromatography is the separation of a mixture into individual components using stationary phase

and mobile phase, liquid chromatography was initially discovered teenth century Link and it was first used separation colored components, "Chromatography" in chromo means colors, graphy means writing, 1st Russian bontanistsmikhail.s chromatographic technique separation of the plant pigments in a pure constituents, the technique of high performance liquid chromatography is so called because of its "improved performance" when compared to classical column chromatography.it also called as a High pressure liquid chromatography



HPLC PRINCIPLE

The principle of separation in normal phase mode and reverse phase mode is

adsorption, when components are introduced into a HPLC column, relative affinity towards the stationary phase adsorbent, two components of there, that are faster, slower components are same affinity stationary phase, the components are separated

TYPES OF HPLC TECHNIQUES

- A. Based on the modes of chromatography
- B. Based on the principle of separation
- C. Based on the elution technique
- D. Based on the scale of preparation
- E. Based on the types of analysis

Normal phase mode

Stationary phase: silica gel

Mobile phase: Hexane, Methyl chloride, chloroform.

Reverse phase m

In a reverse phase technique, a Non-Polar is Stationary Phase, Polar is Mobile phase in a nature, Non-Polar components are retained for a longer time, most of the drugs and pharmaceutical are polar in nature, they are not retained a longer time, different columns used are ODS (Octadecylsilane) or C18, C8, C4, etc.

Ion exchange chromatography

The principle of ion exchange chromatography, which reverse exchange chromatography, in anion exchange resin is used to separate a mixture. Of similar charged ions, for a cations exchange resin is used, for anions exchange resin is used.

Instrumental requirements

Instrumental requirements are used as followed steps

- A. Pumps - Solvent delivery system
- B. Mixing unit, gradient controller and solvent degassing
- C. Injectors - Manual or auto injectors
- D. Guard column
- E. Analytical column
- F. Detectors
- G. Records

Solvent – Pump – Sample injector - Detector - Data system & recorder
Pump

The solvents or Mobile phase must be passed through a column at high pressure at up to 600psi, as the particle size of stationary phase is smaller the resistance to the flow of solvent will be high, hence high pressure is recommended, flow of rates 0.1 to 10mL/min.

Types

- Displacement pumps

- Reciprocating pumps
- Pneumatic pumps

Mixing unit

Mixing unit is mixed solvents in different proportion and pass through the column, there are two types of mixing unit

- Low pressure mixing chamber which uses Helium for degassing solvents.
- High pressure mixing chamber does not require a helium for degassing solvents
- Dynamic mixer which uses magnetic stirrer and high pressure.

Gradient controller

In an isocratic separation, mobile phase is prepared by using pure solvents.

Solvent degassing

Several gases are soluble in organic solvents, when solvents are pumped under high pressure, gas bubbles are formed which will interfere with the separation process, there are following techniques,

- Vacuum filtration
- Helium purging
- Ultrasonification

Injector - Manual or auto injectors

Several devices are available either for Manual or auto injectors of sample, there are following devices

- Septum injectors

It's not common, sample through a rubber septum, it is a high pressure.

- Stop flow

If mobile phase is stopped sample are injected in a valve device

- Rheodyne injector

It otherwise name as a loop valve type, it is most popular injector, this volume injectors are two modes

- Load position
- Inject position

Guard column

Guard column very small quantity of adsorbent of the HPLC technique which decides the efficiency of separation, mode of separation is used

Analytical columns

Analytical column is a heart of chromatography system, it is efficacy of

separation. these are several stationary phase available depending upon the technique. it is usually made up of stainless steel with 1/4 inch external diameter and 4-6mm internal diameter and upto 25cm, they also available in other dimensions, has stainless steel gauze,

Column materials

It is made up of stainless steel, glass, polyethylene and peek (poly ether ether ketone).

Column length: 5cm to 30cm

Column diameter: 2mm to 50mm

Particle nature: Spherical, uniform size, porous material are used.

Surface area: 1gram of stationary phase provides surface area ranging 100-860 sq.m with average of 400sq.m.

Functional groups

It group present in stationary phase depends on the type of chromatographic separation

C18 – OctaDecylsilane column

C8 - Octyl column

C4 – Butyl column

CN- Nitrile column

detectors

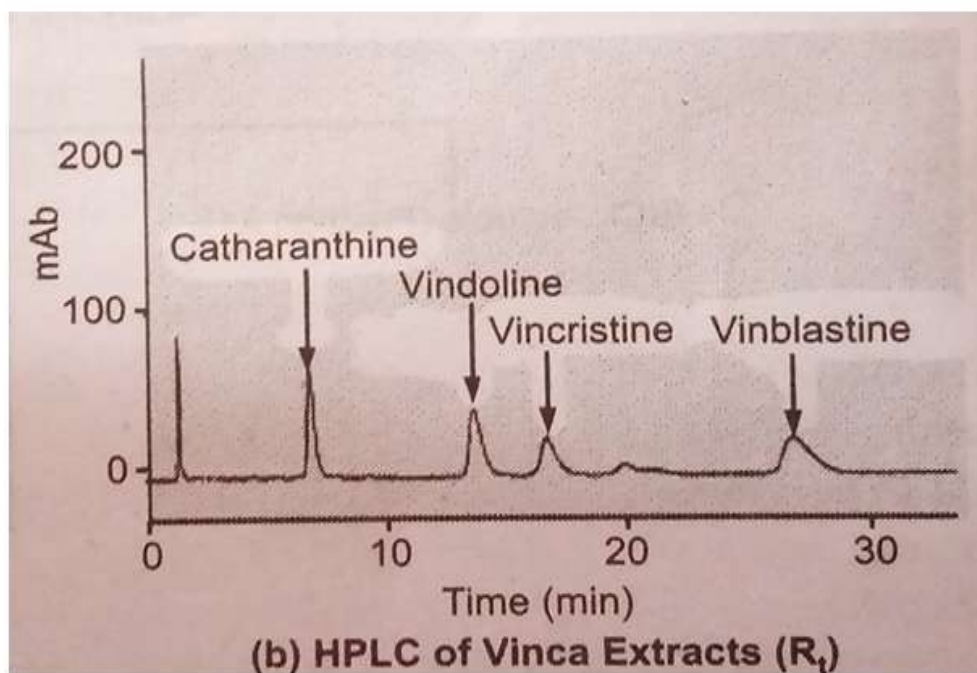
- Refractive index detector
- Fluorimetric detector
- Conductivity detector
- Amperometric detector
- Photodiode array detector

Column: Rp-18e reversed phase chromatolith performance

Mobile phase: Acetonitrile (0.1M), phosphate buffer containing 0.5% glacial acetic acid (21:79), pH=3.5.

Sample preparation

Powdered leaf 5g extract and thrice with 90% ethanol at room temperature, the alcohol extract is filtered and washed with Hexane. The aqueous portion is basified with ammonia to pH 8.5 and extract used chloroform, Chloroform extract is washed water, dried over sodium sulphate and concentrated under vacuum. The residue was redissolved in 10mL methanol



Standard preparation

Stock solutions 0.25mg/mL of each of vincristine, vinblastine, catharanthine and vindole are prepared in methanol and used for preparation of the calibration graphs, linear in the range of the working concentration of each standard.

Detection: 254nm

Flow rate: 1.2mL/min

Retention time: vincristine 16.57min, vinblastine 26.93min

Catharanthine 6.81min, vindoline 13.22min

Therapeutic uses

- ❖ Anti-cancer
 - ❖ Anti hypertensive
 - ❖ Hodgkin's disease of lymphoma
 - ❖ Anti-diabetic
 - ❖ Adulterants and allied drugs
- Eg- catharanthus species viz,
Catharanthus longifolius
- ❖ Vincristine is used treatment of acute lymphocytic leukaemia.

Applications of HPLC

- Qualitative analysis
- Checking the purity of a compound
- Presence of impurities
- Quantitative analysis
- Multicomponent analysis
- Isolation and identification of drugs
- Isolation and identification of mixture
- Stability studies
- Bio pharmaceutical study
- Pharmacokinetics study

Other applications of HPLC

- Clinical application
- Monitoring of hepatic chirosis patient through aquaporin 2 in the urine
- Drug discovery
- Finding New chemical entities (NCE) for adoption as new drug development candidates.
- Chemical development
- Development viable synthetic routes and scale-up process for synthesising Active Pharmaceutical Ingredients (API)
- Pharmaceutical development

Developing dosage form with optimised delivery system

- Biochemical genetics
- Bio synthesis study in detection of Biogenetic intermediates and enzymes involved.
- Petro chemical
 - Protozomics

Structure proteomics

- Organella composition
- Subprotozome isolation
- Protein complex

II. CONCLUSION

HPLC technique is alkaloids in vinca as a used vincristine vinblastine are a anti cancer and anti hypertensive and Hodgkin's disease. HPLC is produce a extremely pure compounds, it are used in a analytical technique and laboratories, clinical, drug discovery. it are use both a qualitative & quantitative analysis compounds, HPLC demirts only high cost.

REFERENCE

- [1]. Dr.S.Ravi Shankar 5th edition 18-1 to 18-15
- [2]. Analysis of catharanthus roseus alkaloids by HPLC, published online: 13 March 2007 author stevehisiger: Mario jolicoer.
- [3]. Determination of vinca alkaloids in periwinkle plants using HPLC-ECD -A Thermo Fischer scientific board.
- [4]. Castle, M, C, (1989), plant alkaloids, The vinca alkaloids, cancer growth prog., 10, 147-131.
- [5]. Budman D, R, (1992), New vinca alkaloids and related compounds, semin, 19, 638-645.