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Extraction Of Vindoline From Catharanthus Roseus And Instrumental Model Of Automatic Column Chromatography By Using PLC

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Abstract: Alarming rise in the incidence of Cancer has become serious concern for scientists across the globe. Cancer caused by rough cell growth has always been a topic of research in medicinal herbs. The project involves extraction of vindoline from a herb catharanthus roseus. Vindoline is a bio-active compound and is being used for the treatment of cancer. Vindoline is currently extracted by chromatographic purification. This work involves further purification of vindoline by recrystallisation using methanol as solvent and thus vindoline produced meets the stringent requirements of pharmaceutical industry. This compound is being extracted from the herb using various chemicals[1]. We have used PLC Logic ladder diagrams and SCADA software to find a suitable automation for automatic column chromatography.
Keywords: vindoline, catharanthus roseus, methanol recrystallization, PLC logic ladder diagram.

Introduction

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer cells can spread to other parts of the body through the blood. A Carcinoma **-** cancer that begins in the skin or in tissues that line or cover internal organs. There are a number of subtypes of carcinoma, including adeno carcinoma, basal cell carcinoma, squamous cell carcinoma, and transitional cell carcinoma. Vindoline is a bioactive compound extracted from the aerial parts of *catharanthus roseus* which can be used in the treatment of cancer. This plant also yields sub constituents such as vinblastine,vincristine and ajmalicine which are also important in the treatment of cancer[1].
catharanthus roseus

Family: Apocynaceae [2]

One of the few medicinal plants which has found mention in the folk (medicinal literature-2nd century B.C) an alkaloid derived from aerial part of periwinkle.

Common Name: Periwinkle, Nityakalyani [2]
Chemical Constituents: Vincristine, Vinblastine, Vindoline and Ajmalicine[2]

Applications:

- Decoction of young leaves used for stomach cramps.

- Used in Pharmacology.

- Infusion of leaves used for treating menorrhagia.

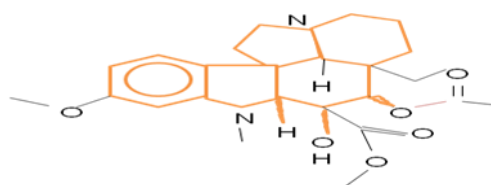The anti-cancer drugs namely Vincristine and Vinblastine are alkaloids produced from catharanthus roseus.

vindoline

Composition: Availability[2] of Vindoline component in

- Roots is up to 0.5%

- An aerial part is 0.2–1%.

Temperature should be maintained below 50°C.The structure of vindoline is represented by (Figure- 1).

Figure 1: Structure of Vindoline

Applications:

- Its ability to reduce blood pressure

- Used as Anti-cancer drugs

Procedure

Preparation of Extract

**Material balance: Table 1: Material Balance for Vindoline**

Stage	Description	Input	Output	Accumulation	waste	Yield
1	100 gm wet leaves taken for drying we got 34 gm of dried powder	34 gm	10 gm	Nil	14gm	29.4%
2	200gm of Al_2O_3 packed,	10 gm	7gm	Nil	3gm	70%
3	Vindoline separation from the other constituent using acidification and layer separation	7gm	3gm	4gm	Nil	42.8%
4	Recrystallisation	3gm	1.8gm	Nil	1.2gm	60%

Solvent Cost involved in extraction: Table 2: Solvent Cost

Description (Input for 100gm)	Required QTY	Unit	Price in Rupees
1. Methanol	1400	Ml	470.00
2.Dichloride methane　(MDC)	400	Ml	323.00
3. NH_4OH 25%	20	Ml	12.00
4. Anhydrous sodium sulphate	10	Gm	10.00
5. Aluminum oxide	200	Gm	150.00
6. Chloroform	100	Ml	70.00
7. tartaric acid	1	Gm	3.00
Total			1038.00

About 34 grams of the sample leaves was weighed and taken in an iodine flask. Ethanol was added to it in the ratio of 1g: 10ml. 95% methanol was used for extraction. 340ml of methanol was added and extracted in shaker for 48hours, under 160rpm. This gave the crude extract a dark green color. 370ml of crude extract was obtained after vacuum filtration until suck dry. The extraction process done is shown in (Figure- 2). Material balance and solvent extraction is shown (Table- 1 & 2)

Figure 2: Preparation of Extract
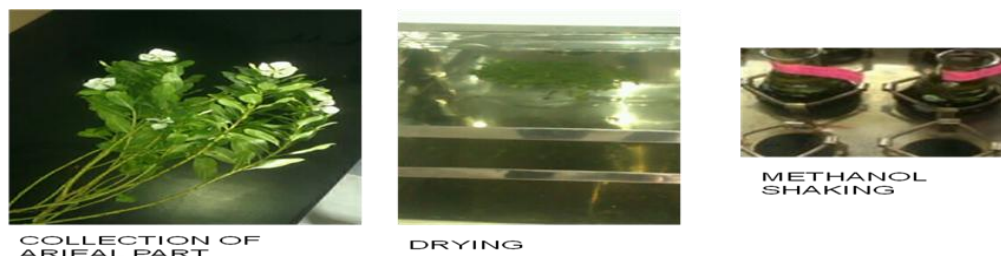
Solvent Evaporation

The obtained extract was evaporated under 500mmHg at below 50°C to give residues. To evaporate the extract and also to recover the methanol a vacuum distillation experimental setup was done. It included a condenser, round bottom flask, a connector, recovery flask, vacuum pump with pressure controller and a muffle furnace[3].

The extract was dried and methanol was removed completely. 300ml of methanol was recovered. Hence about 88% of used methanol was recovered using this setup. From the above residue TLC test was done using TLC analysis procedure[4]. We obtained product spot in eluted region. So, extraction of compound was completed. Photocopy of the filtration and evaporation process is shown in (Figure- 3).

Figure 3: Photocopy Of Evaporation And Filtration Using Vaccum Distillation

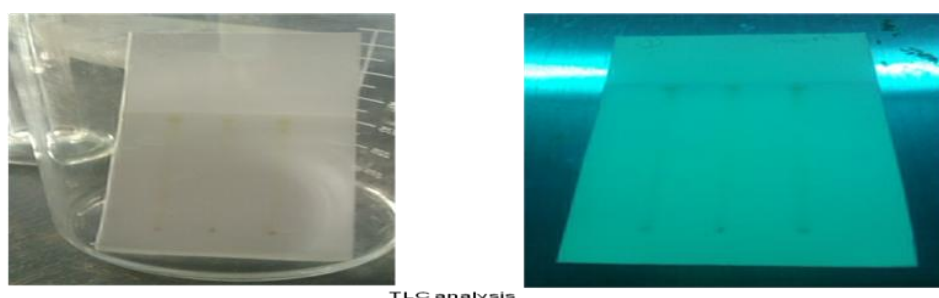Collection of residue by Vacuum distillation

Chromatographic Screening:

Sufficient methanol was added to chromatographically screen the methanol extract for the presence of alkaloids based on TLC analysis[4]. The remainder of the methanol extract was then dried at 50°C.

Column Packing Procedure:

Figure 4: TLC Analysis

TLC analysis

The column was cleaned and little amount of non-adsorb cotton was placed at the bottom of the column. A slurry of aluminum oxide with chloroform was made and slowly charged into column side walls to avoid cavitations. The bottom valve was opened while charging the slurry. The flow rate was fixed as per column packing height and diameter. Then chloroform was eluted up to the level of packing. The crude material was then charged at the top of packing. Then immediately the non-adsorb cotton was placed to avoid the bed disruption. The TLC analysis test is shown in (Figure- 4). Then methanol screen was eluted and the bottom fraction was collected as per TLC analysis[4].
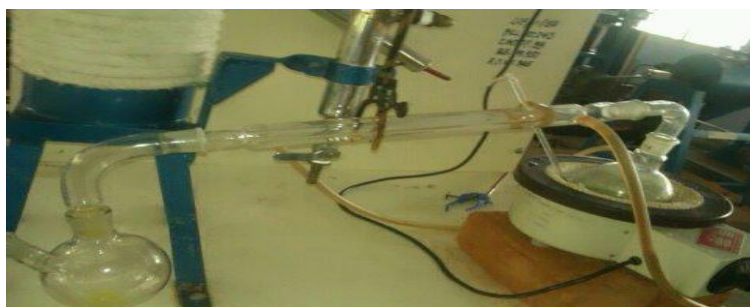
Separation of Vindoline:

5 ml of 2% tartaric acid was used to acidify the dried extract[5]. Equal volume of dichloromethane was added into the acidified extract and this was used to extract chlorophyll, neutral alkaloids and other neutral compounds. The solvent was filtered off. The tartaric acid layer was removed and adjusted to pH 5.9 (Figure- 5 & 6) with 25% NH_4OH. Most neutral alkaloids remained in the organic phase while most other alkaloids were isolated after basification. The two separated liquid phases were further extracted to obtain individual alkaloid rich extracts. The remaining dichloromethane layer was washed three times with distilled water. Water layer was decanted and the organic layer was dried over anhydrous Sodium sulphate. This was then filtered and concentrated in vacuum at 50 ^{o}C till dryness. The extract was then dissolved in 75 ml methanol for chromatographic analysis.

Figure 5: Ph Adjustment

Figure 6: Seperation of Vindoline

Recrystallisation Of Vindoline:

Figure 7: Stages of Recrystallization

The crude was dissolved in 8 ml of methanol and 1 g of activated charcoal was added and then stirred for 10 min. The solution was filtered through Buchner funnel followed by filtration through 0.45micron setup. The clear solution was taken in a round bottom flask in water bath and was shaken for 2 hours at 0-5°C under 160 rpm speed. The round bottom flask was maintained at the desired temperature for two hours. On cooling vindoline forms crystals and separates at the bottom of the flask. Crystals are filtered through Buchner filter and

the slurry is charged into the drier to get dry powder[6]. The drier is maintained for six hours under vacuum below 40°C. The filtrate is preserved for yield estimation. Dried sample is sent for analysis[7].The various stages of recrystallisation are shown in (Figure- 7). The HPLC result before and after crystalliasation are plotted graphically in (Figure- 8 & 9).

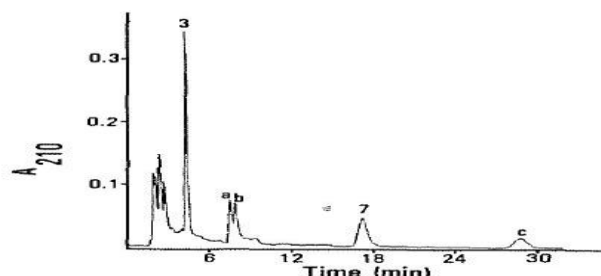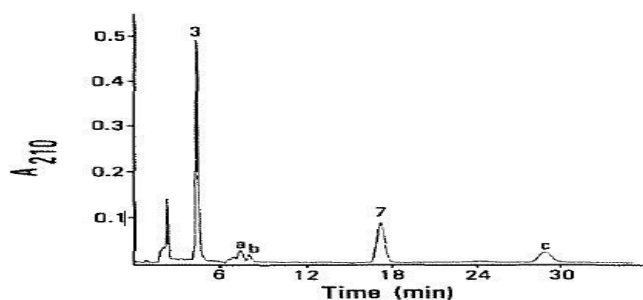**Figure 8-Graph 1: HPLC result before Recrystallisation**

Figure 9-Graph 2: HPLC result after Recrystallisation

From the graphs it was found that recrystallisation is more efficient. The purity of vindoline obtained from recrystallisation is approximately 92% and before recrystallisation it is 85% pure.

Instrumental Model Of Automatic Column Chromatography By Using PLC:

Automation of the chromatography column is established using SCADA software[7]. Column packing is done manually to prevent cavitations. Elution is a major factor in manual packing since the eluent may overflow resulting in emptying of the mobile phase at the top of the column. Hence automation using programmable logic controller is primarily employed to control the flow rate of the mobile phase. The instrumental model of automatic column chromatography is shown in (Figure- 10).

Figure 10: Automatic Column Chromatography

Software details
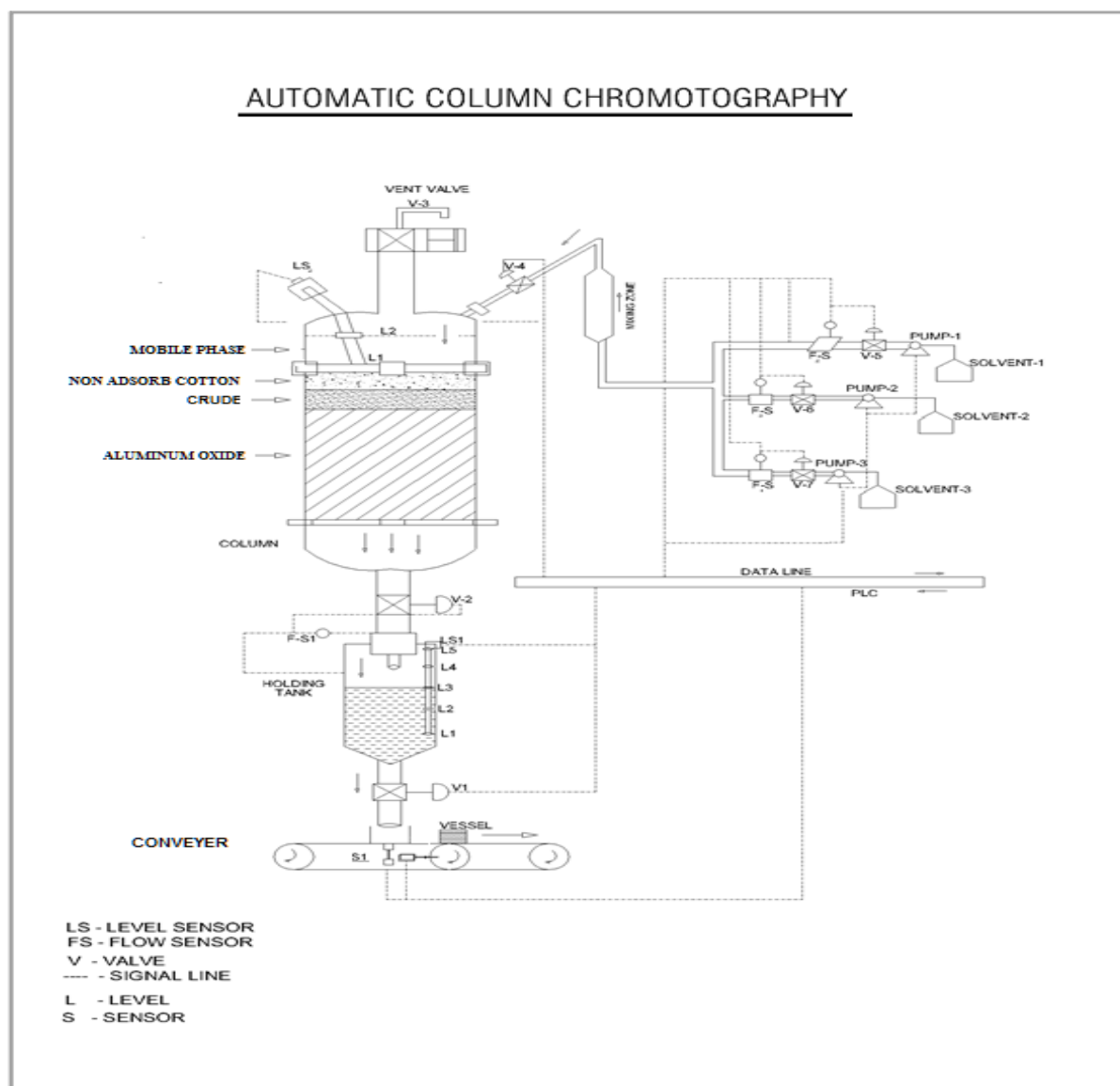
Software : RS Logix 500[7]
Supporting software : RS Linx gate way
Configured drivers: EMU 500-1 SLC 500 (DH 485)
Controller properties: Bul 1761 micro logix 1000 DH-485 / HDS Leve
Controller communication driver : EMU 500-1
1761 micro process 1000

AUTOMATIC COLUMN CHROMOTOGRAPHY

Ladder diagram operation:

Procedure:

Ladder 0000:Emergency run:

The purpose of this ladder is to operate in safe mode. One NO, NC input and Output us. NO (I: 0/0) was used to start the overall programs. NC (I: 0/1) was used to stop all the programs. Output (O: 0/0) was run as per input signal for all the section of the column operation program[7].

Ladder 0001-0006: Operation of fraction collection (bottom of the column)

The run output is taken to operate the bottom section of the column When the vessel sensor (I: 0/2) does not sense, the holding tank valve is switched on as per timer instruction followed by binary output (B: 0/0) then conveyer motor (O: 0/1) starts to move the vessel (beaker) to the right position. Otherwise output of the conveyer motor automatically goes off. When the vessel sensor is switched on the delay timer (TON) is switched on (T4:0) as per the preset value (5 sec). After the accumulation of preset time (5sec) the done (DN) signal is displayed (T4:0/DN), then binary output (B$_3$: 0/1)is displayed, when timer binary (B$_3$: 0/1) is switched on then as per requirement of level sensor (level 1, 2, 3, 4, 5) the holding tank valve opens (O: 0/2).When holding tank valve is switched on, then delay timer TON (T: 1) is set on as per the time set. After accumulation of time the done bit is turned on and it turns on the binary output of conveyer[7].The ladder diagrams are given in (Figure- 11 & 12).

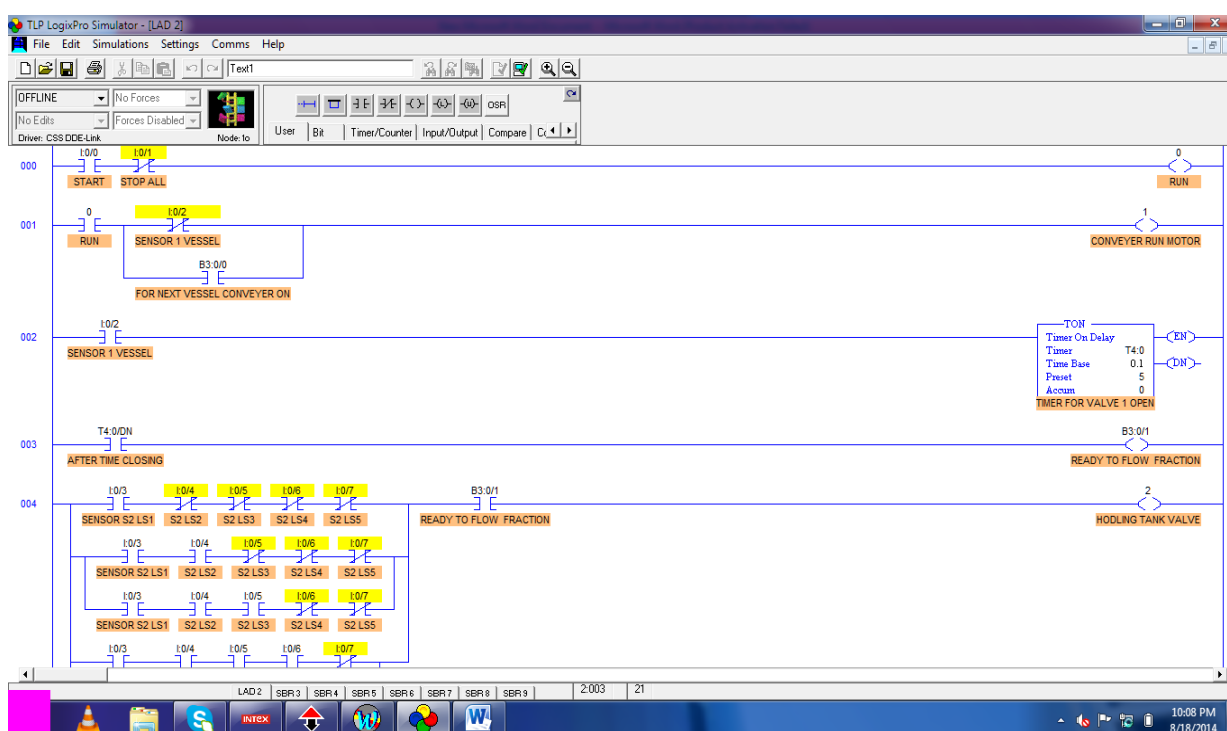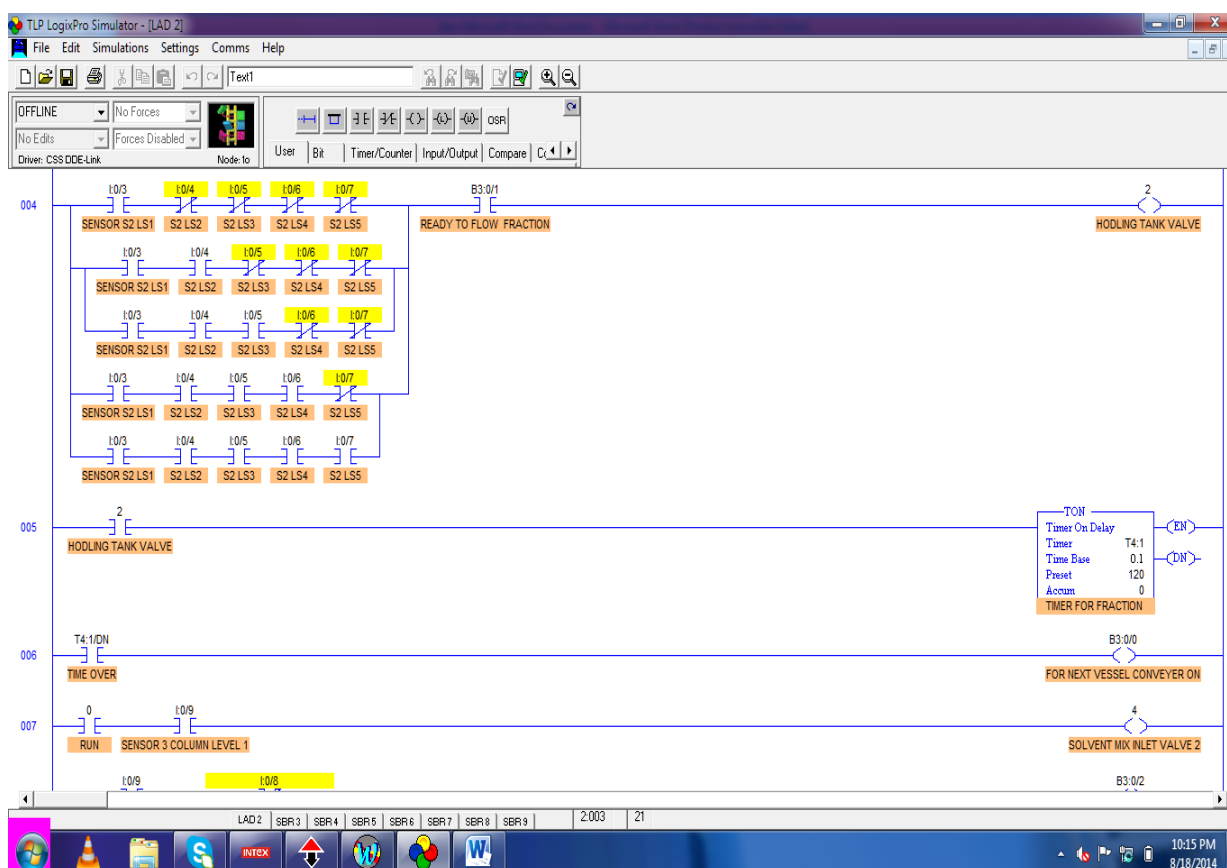**Figure 11: TLP Logixpro Simulator(Lad 2)**

Figure 12: TLP Logixpro Simulator (Lad 2)

Ladder 0007-0011:Operation of mobile phase loading (top of the column)

The run output is taken as input to operate the top section of the column .When the column level sensor 1 (I: 0/9) is turned on and column level sensor 2(I: 0/8) does not sense then pump ,binary bit(B$_3$: 0/2) is set on. When the pump binary is on and required motor switch is in on condition (phase 1, 2, 3) then correspond pump will get on.

Figure 13: TLP Logixpro Simulator (Lad 2) :
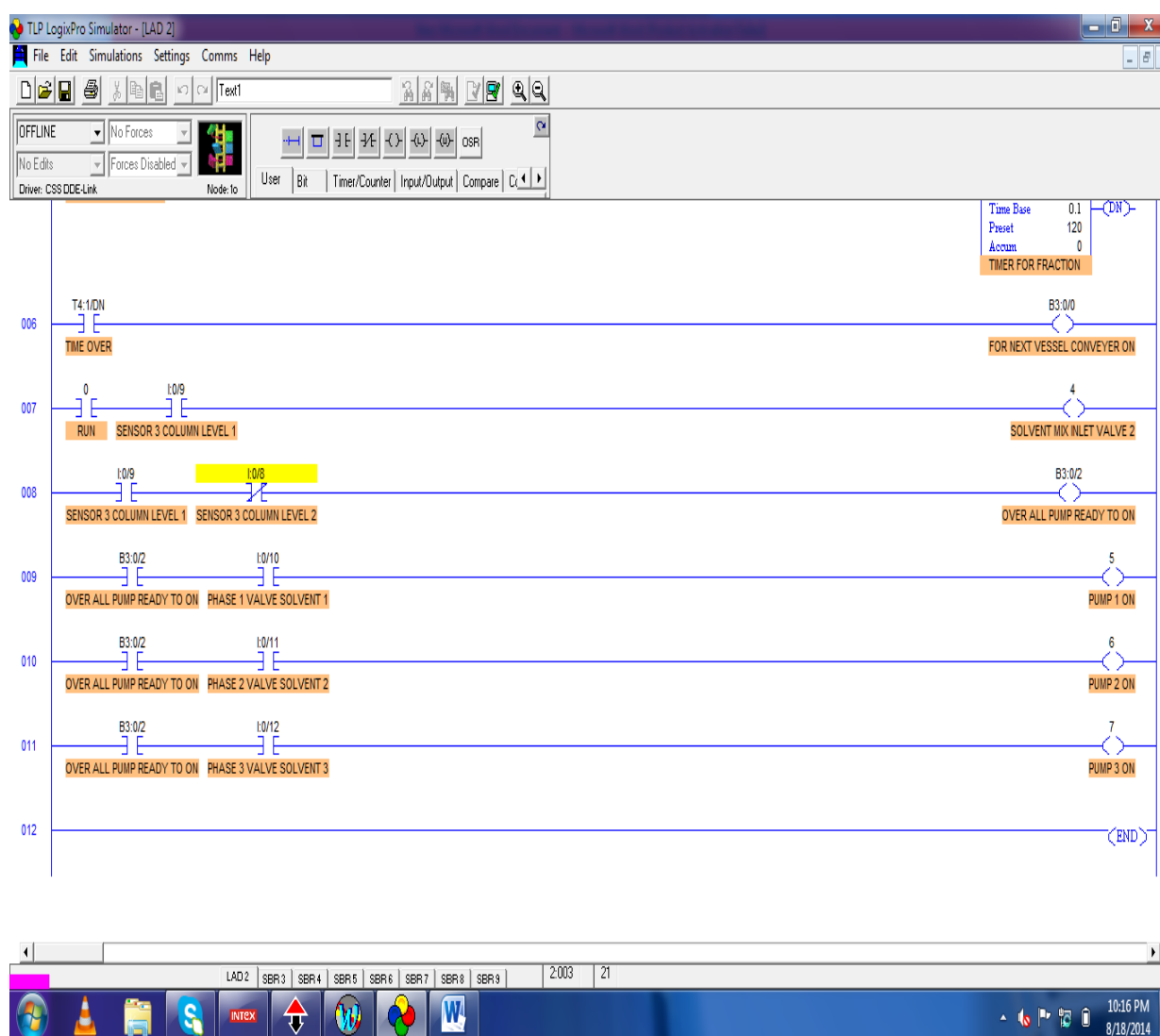
TLC analysis

Method: spot   pointing (Figure- 14)

1.   Reference(material if not available check with reference paper)
2.   Co spot
3.   Required analysis spot(extracted vindoline)

 All samples are diluted with MDC for sample preparation
 Spot pointing by using capillary tube

Stationary phase:

 Silica gel GF 254 TLC plate (readymade)[8]

Mobile phase:

MDC: methanol
 8   :   2

NOTES: After the TLC elution, need to check under the UV light[9] (we used laminar air flow)
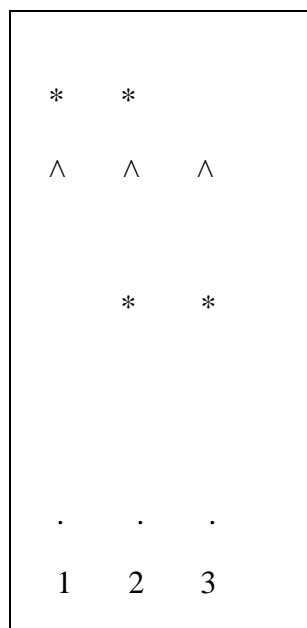
Figure 14: TLC Analysis

Where

∧   ----> vindoline

*   ----> impurity

1, 2, 3 ---->

as per spot pointing procedure

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 *    *

 ∧    ∧    ∧




      *    *




 .    .    .

 1    2    3
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Conclusion

The extraction of vindoline from _catharanthus roseus_ was successful and was identified using HPLC and TLC. Recrystallisation proved to increase the quality of vindoline and thus the suitable model of automatic column chromatography was successfully done using the PLC logic diagrams.

References

1. Sukhdev Swami HandaSumanPreet Singh KhanujaGennaro Longo DevDuttRakesh"Extraction Technologies for Medicinal and Aromatic Plants" Online book : International Centre for science and high technology. 2008.
2. Schmelzer, G.H. &Gurib-Fakim, A.   [CD-Rom].. PROTA, Wageningen, Netherlands Prota 11(1): Medicinal plants/Plantesmédicinales 1 Catharanthus roseus (L.) Record display, Schmelzer, G.H., 2007.
3. ZHANG Jing, LONG Xiao-hua, LIU Ling, LIU Zhao-pu "Optimizing the Extraction Process of Four Important Alkaloids from Catharanthus roseus by Ultrasonic Method. (English)." ISSN: 1001-6880 Accession Number: 67308558 Vol. 23 Issue 2, p309-313. 5p Apr2011,.
4. N.L. Magagula , V. Mohanlall and B. Odhav, Department of Biotechnology and Food Technology, Durban University of Technology, South Africa , "Optimized Thin Layer Chromatographic Method for Screening Pharmaceutically Valuable Alkaloids of Catharanthus roseus" (Madagascar Periwinkle) ISSN 2305-3925 Dec-2012.
5. Khaled A Shams, Naglaa M Nazif, Nahla S Abdel Azim, Khaled A Abdel Shafeek, Mostafa M El-Missiry,   Shams I Ismail, and   Medhat M Seif El Nasr.   "Isolation and Characterization of Antineoplastic Alkaloids from Catharanthus Roseus L. Don. Cultivated in Egypt" Mar 7, 2009.
6. http://www.foothill.edu/psme/armstrong/recrystallization.shtml column packing procedure.
7. Allen-Bradley AB PLC manual (SLC 500)SLC 500 andMicroLogix 1000 Instruction Set January 1996 Copyright 1998 Rockwell International Corporation.
8. SetiyoGunawan, SuryadiIsmadji, Yi-Hsu Ju "Design and operation of a modified silica gel column chromatography" Journal of the Chinese Institute of Chemical Engineers November 2008.
9. http://orgchem.colorado.edu/Technique/Procedures/Columnchrom/Procedure.html column packing procedure .

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