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Large-scale separation of clavine alkaloids from *Ipomoea muricata* by pH-zone-refining centrifugal partition chromatography☆

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ABSTRACT

Centrifugal partition chromatography in the pH-zone-refining mode was successfully applied to the separation of alkaloids, directly from a crude extract of *Ipomoea muricata*. The experiment was performed with a two-phase solvent system composed of methyl *tert*-butyl ether (MtBE)–acetonitrile–water (4:1:5, v/v) where triethylamine (10 mM) was added to the upper organic stationary phase as a retainer and trifluoroacetic acid (10 mM) to the aqueous mobile phase as an eluter. From 4 g of crude extract, 210 mg lysergol and 182 mg chanoclavine were obtained in 97% and 79.6% purities. Total yield recovery was >95%. Isolated alkaloids were characterized on the basis of their ^{1}H, ^{13}C NMR and ESI-MS data.

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1. Introduction

Ipomoea muricata synonyms *I. turbinate*, *Calonyction muricata* belongs to the family of the Convolvulaceae. It is a creeper and widely grows in all places. The plant is commonly known as morning glory. Flowers are purple or white up to 8 cm diameter. Fruits are pendulum shape capsules. Seeds are commonly known as “Kaladana” in trade and are being used as purgative in India and Pakistan [1]. The seeds of ipomoea have been reported to be good source of insect metamorphic hormones and clavine alkaloids, which find a wide variety of biological activities [2]. The seeds have been reported to contain about 0.49% of alkaloid of which lysergol constitutes ~53% and chanoclavine ~37% (Fig. 1) of the total alkaloids [1]. Lysergol has been used as hypotensive, psychotropic analgesic and uterus and intestine stimulating drug [3]. Recently we invented the novel bioenhancing potential of lysergol, which acts as bioavailability facilitator for antibiotics and helps in transferring the antibiotics across the membrane for better efficacy on the target site [4]. Lysergol is also used as starting material for the synthesis of modern anti-Parkinson drug “Cabergoline” [5]. On the other hand chanoclavine is known as a precursor for the biosynthesis of pharmacologically active ergot alkaloids [6] and can be utilized, inter-alia, for the production of pharmacologically effective compounds [7].

Although there are several patents on the isolation process of lysergol from the seeds of ipomoea [3], but due to heat and light sensitivity of lysergol, its economical and quantitative isolation has always been a great problem. This article shows that this problem was easily solved by means of centrifugal partition chromatography (CPC) when used in the pH-zone-refining mode [8].

Centrifugal partition chromatography (CPC) which utilizes centrifugal force to enhance phase separation, provides a new dimension in the area of separation science [9]. CPC is based on liquid–liquid partitioning and is an excellent alternative to circumvent the problems associated with solid phase adsorbents to preserve the chemical integrity [10] of mixtures subjected to fractionation and isolation [9]. In a biphasic solvent system, one liquid phase is made mobile while the other one is made stationary inside the column by a constant centrifugal force field. The column is built as a multiple disks stack in which partition cells are engraved and a careful selection of biphasic solvent system may allow separation of compounds having close structural resemblance [11]. Since there is no problem due to saturation of the solid-stationary phase, CPC is gaining importance as a preparative separation method [8].

pH-zone-refining centrifugal partition chromatography was developed by Ito and coworkers [12–16] as a preparative purification method for the separation of compounds whose electric charge depends on pH-value. The method enables separation of organic acids and bases into a succession of highly concentrated rectangular peaks with minimum overlap that elute according to their pKa values and hydrophobicities. pH-zone-refining CPC

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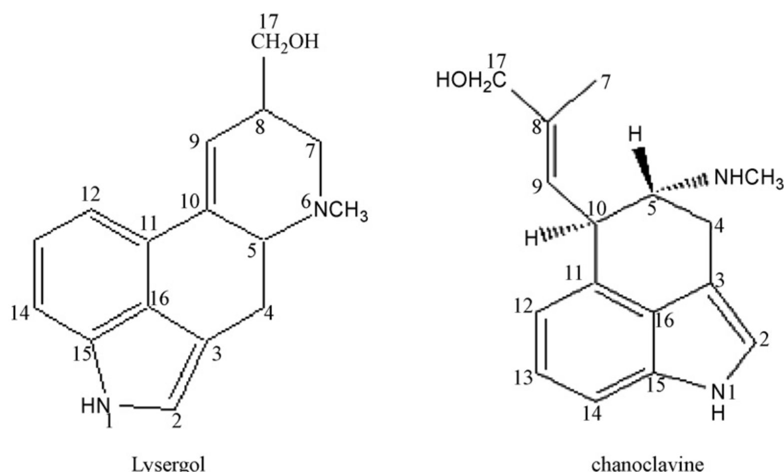


Fig. 1. Structure of interesting alkaloids found in the seeds of *Ipomoea muricata*.

provides many advantages over the conventional CPC such as more than 10-fold increase in the sample loading capacity, high purity and high concentration of fractions, concentration of minor constituents and minor impurities, etc. The method has been successfully used for the separation of a wide variety of natural products [8,17], alkaloids [8,12,15,18], structural [19] and geometrical [20] isomers, acidic [14,21] and basic [22] amino acid derivatives, synthetic colors [14,23] and chiral compounds [24]. The present paper reported successful multigram preparative pH-zone-refining CPC separation of two alkaloids, lysergol and chanoclavine from the crude alkaloid extract of *I. muricata* seeds.

2. Experimental

2.1. CPC apparatus

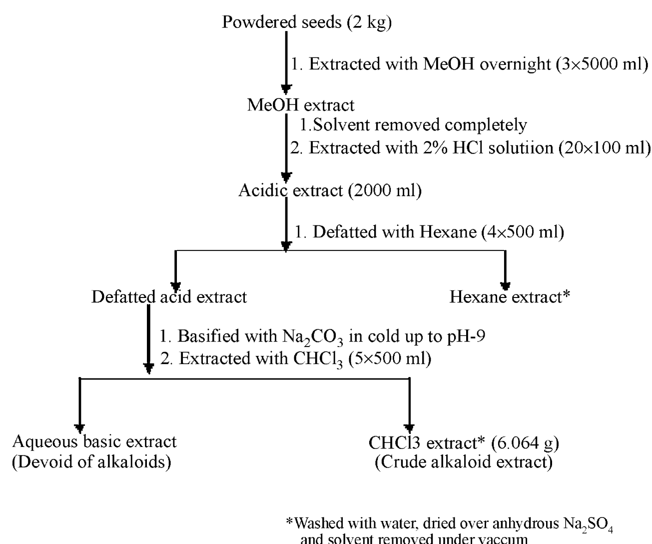
The separations were performed using Kromaton Technologies (Angers, France) apparatus FCPC with a rotor of 20 circular partition disks (1320 partition cells, column capacity 200 ml). Rotation speed could be adjusted from 200 to 2000 rpm, producing a centrifugal force field in the partition cells of about $120 \times g$ at 1000 rpm and $470 \times g$ at 2000 rpm. The column was connected to injector or to the detector through high pressure rotary seals. A four port valve installed on the CPC, allows its operation both in ascending and descending modes. The CPC was connected to a Waters (USA) 2525 modular gradient pump. The samples were introduced into the CPC column – after equilibration of the two phases – via a Rheodyne injection valve (ALTech Association, Deerfied, IL, USA) equipped with 5 and 20 ml sample loops, respectively. The effluent was monitored with Waters PDA 2996 detector equipped with a preparative flow cell set at wave length 225 nm. Fractions were collected manually and pH was checked with Consort pH Meter model C861 (Japan). Purity of the isolated compounds was checked on a Chromolith performance RP-18e column (100 mm × 4.6 mm I.D., Merck), Dionex Summit P680 HPG preparative binary gradient pump (Sunnyvale, CA, USA) equipped with Dionex PDA-100 detector. The experiments were conducted at room temperature (25 ± 1 °C).

2.2. Reagents

Methyl-*tert*-butyl ether (MtBE) (HPLC grade) was purchased from Spectrochem (Mumbai, India) and acetonitrile (CH_3CN, GR), methanol (MeOH, GR), water (H_2O, Deionized), sodium dihydrogen phosphate (NaH_2PO_4, AR grade), triethylamine (TEA, extra pure), and trifluoroacetic acid (TFA, extra pure) were purchased from Merck (Worli, Mumbai, India).

2.3. Extraction of alkaloid and sample preparation

The seeds of *I. muricata* (Kaladana) were purchased from the local market, Lucknow, India. Extraction of alkaloid from the powdered seeds was carried out according to the following flow chart.



The two separations were carried out by preparing sample solutions of 500 mg and 4 g of crude alkaloid extract ($CHCl_3$ extract) in 5 and 15 ml of the solvent mixtures consisting of stationary phase.

2.4. Preparation of solvent system

The biphasic solvent system used in the present study was consisted of MtBE–CH_3CN–water (4:1:5, v/v). The solvent mixture was vigorously shaken and then allowed to settle until the phases became limpid. The upper organic phase was made basic with TEA (retainer) to obtain a final concentration of 10 mM and trifluoroacetic acid (eluter) was added to the lower aqueous mobile phase to obtain a final concentration of 10 mM.

2.5. Separation procedure

Keeping the rotor speed at 400 rpm, the column was first filled with basic organic layer containing TEA (retainer) at 10 mM as sta-

Table 1
Experimental conditions for two separations (500 mg and 4 g) of crude alkaloid extract of *Ipomoea muricata*.

	Separation 1	Separation 2
Sample (injection volume)	500 mg (5 ml)	4 g (15 ml)
Apparatus	FCPC Kromaton with 200 ml rotor	FCPC Kromaton with 200 ml rotor
Elution mode	Descending	Descending
Biphasic solvent system	MtBE–CH_3CN–water (4:1:5)	MtBE–CH_3CN–water (4:1:5)
Stationary phase (retainer)	Organic (TEA10 mM)	Organic (TEA10 mM)
Mobile phase (displacer)	Aqueous (TFA10 mM)	Aqueous (TFA10 mM)
Rotation (rpm)	1250	1250
Flow-rate (ml/min)	3	3
Back pressure (bar)	53–55	53–55
Detection	UV 225 nm/pH monitoring	UV 225 nm/pH monitoring
Fraction time (min)	2	2

tionary phase at a flow-rate of 6 ml/min. Then the rotor speed was increased to 1250 rpm and aqueous phase containing trifluoroacetic acid (eluter) at 10 mM was pumped into the inlet of the column at a flow-rate of 3 ml/min in the head-to-tail elution mode (descending mode). This increased the back pressure from 33 to 54 bar, causing displacement of about 15–25% of stationary phase. Once equilibrium was maintained between mobile and stationary phase, the sample was injected through the sample port. The effluent from the outlet of the column was continuously monitored with a Waters PDA 2996 detector at 225 nm and the evolution of the pH was continuously monitored using a pH meter. After elution of the desired compounds, rotor speed was further reduced to 400 rpm and column was washed with a mixture of water:methanol (1:1) in ascending mode at a flow-rate of 6 ml/min until complete stationary phase was pumped out. On completion of the experiment (as per advice of the manufacture) the column was filled with a mixture of water:methanol (1:1).

2.6. Characterization of the alkaloids and their analysis

All the fractions were checked by TLC on Merck silica gel $60F_{254}$ ready made plates developed with $CHCl_3$:MeOH (85:15). The fractions of interest were identified on the basis of their ^1H, ^{13}C NMR spectroscopic data in pyridine. The 300 MHz ^1H and 75 MHz ^{13}C NMR spectra were recorded on Brucker 300 spectrometer. The isolated alkaloids lysergol and chanoclavine were analyzed by HPLC (DIONEX) with a Chromolith performance RP-18e column (100 mm × 4.6 mm I.D.) (MERCK) at 225 nm with acetonitrile–0.01 M sodium dihydrogen phosphate buffer containing 0.2% trifluoroacetic acid (15:85, v/v, pH 2.5) as mobile phase with 1 ml/min flow-rate.

3. Results and discussion

pH-zone-refining CPC has been successfully applied to the separation of alkaloids [8,12,25]. For the successful separation of alkaloids in the present application, a suitable two-phase solvent system was necessary, which should provide ideal partition coefficient (K) values in both acidic ($K_{acid} \ll 1$) and basic ($K_{base} \gg 1$) condition as well as good solubility of the sample in the solvent system [26]. First, we tried a two-phase binary solvent system consisted of MtBE–water, which has been used for the separation of various kind of compounds [8,23]. Although this solvent system produced suitable K values, but due to the poor solubility of alkaloids in this solvent system, it could not be used. By adding CH_3CN to the above solvent system, solubility of the sample was substantially improved and the solvent system was optimized by selecting MtBE–CH_3CN–water (4:1:5, v/v). In order to investigate the preparative aspect of pH-zone-refining centrifugal partition chromatography, two separations of *I. muricata* alkaloids with an increasing mass of injected samples 500 mg and 4 g were carried out in reverse-displacement mode using 10 mM TEA (as retainer) in the upper organic stationary phase and 10 mM TFA (as eluter) in lower mobile phase. Experimental details are listed in Table 1.

Fig. 2A shows a typical pH-zone-refining centrifugal partition chromatogram obtained from the separations of 500 mg of crude alkaloid extract of *I. muricata* seeds by the reverse-displacement mode with the 10 mM TEA (as retainer) in the upper organic stationary phase and 10 mM TFA (as eluter) in lower mobile phase. Alkaloids were eluted as irregular rectangular peak where absorbance plateaus were observed at retention times of 40–46, 46–48 and 48–54 min. The measurement of the collected fractions also revealed three flat pH zones, I–III which, respectively correspond to the above absorbance plateaus, suggesting the successful separation of chanoclavine at retention times 40–46 min in pH-zone I, mixture of chanoclavine and lysergol at retention times 46–48 min in pH-zone II and lysergol at 48–54 min in pH-zone III. Considerable amounts of impurities were eluted in the front and the back of the main peaks, forming multiple peaks.

Fig. 2B shows similar pH-zone-refining centrifugal partition chromatograms obtained from the separation of 4.0 g of the same crude alkaloid extract of *I. muricata.* The observations clearly showed that an increase in the sample mass causes a lengthening of the elution time for each component of the mixture, without affecting the transition zones. Therefore, the period of time during which pure compounds are eluted are longer, relative to the over all separation time. Based on HPLC analysis and the elution curve of the pH-zone-refining centrifugal partition chromatography, all

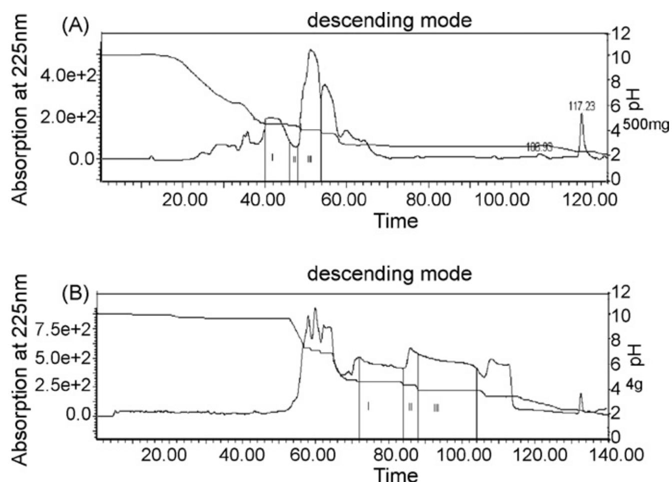


Fig. 2. Separation of lysergol and chanoclavine from the crude alkaloid extract of *Ipomoea muricata* seeds by pH-zone-refining CPC. Solvent system: MtBE–CH_3CN–water (4:1:5, v/v), 10 mM TEA in the upper organic stationary phase and 10 mM TFA in lower mobile phase; sample size: (A) 500 mg and (B) 4.0 g; flow-rate: 3 ml/min; detection: 225 nm; revolution speed: 1250 rpm; retention of stationary phase: 70–80%.

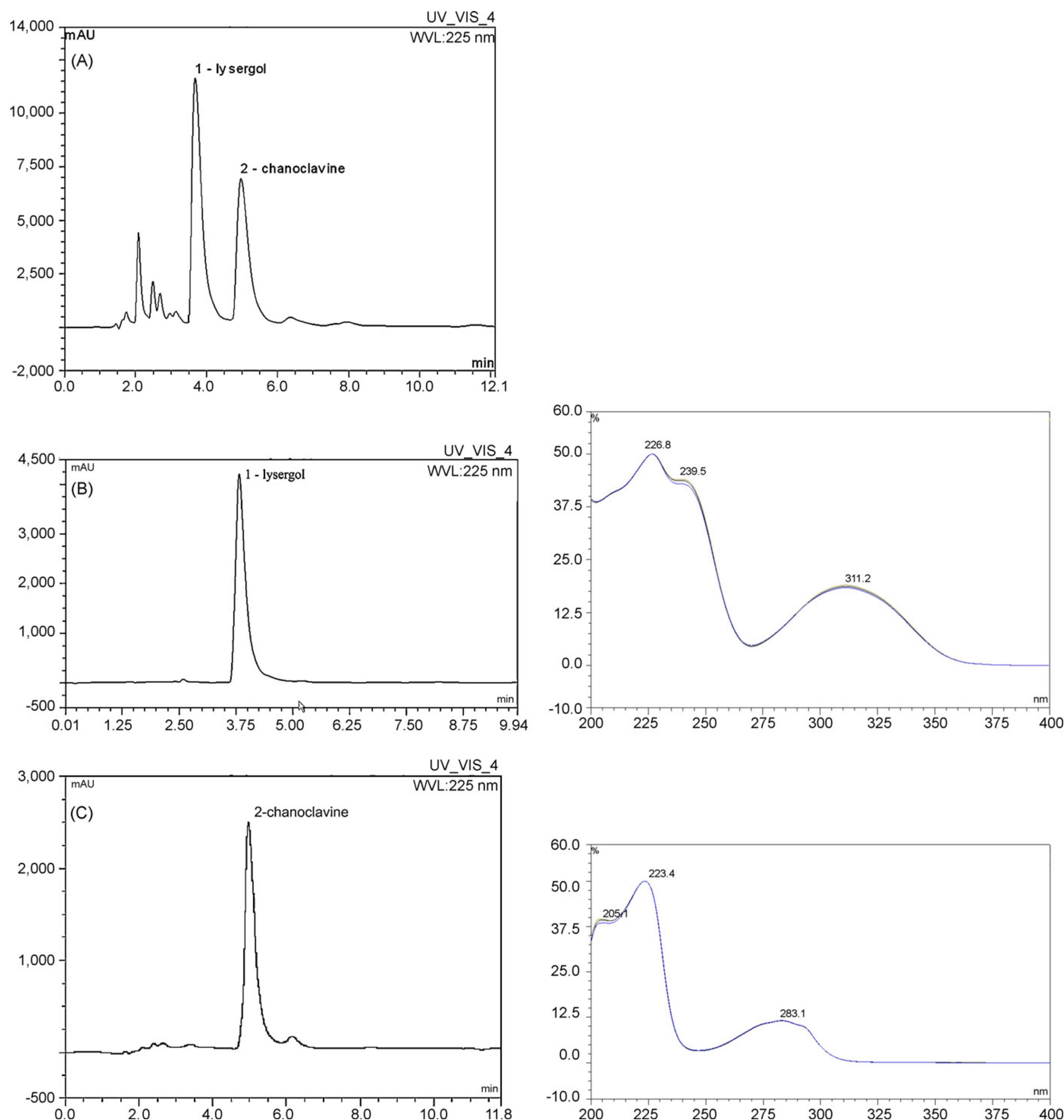


Fig. 3. HPLC analyses of original sample and two pH plateaus fractions from pH-zone-refining CPC separation with 4 g sample size (Fig. 2B). Column: Chromolith performance RP-18e (100 mm × 4.6 mm I.D.); column temperature: 25 °C; mobile phase: acetonitrile–0.01 M sodium dihydrogen phosphate buffer containing 0.2% trifluoroacetic acid (15:85, v/v, pH 2.5); flow-rate: 1.0 ml/min; UV detection wavelength: 225 nm; injection volume: 5 μl. (A) Crude alkaloid extract: peaks 1-lysrgol and 2-chanoclavine (B) peak III in Fig. 2B (lysergol); (C) peak I in Fig. 2B (chanoclavine).

collected fractions were combined into different pooled fractions. Injection of 4 g crude alkaloid extract having 53% lysergol and 37% chanoclavine (Fig. 3A) resulted in the isolation of 210 mg lysergol (pH-zone III in Fig. 2B) with 97% purity (Fig. 3B) and 182 mg chanoclavine (pH-zone I in Fig. 2B) with 79.6% purity (Fig. 3C) in one step separation. The purity enhancement may also be explained by considering that each alkaloid in the column behaves as a retainer for the alkaloid just behind. In this way an increase in the sample mass allows a better organization of the repartition of the alkaloids in the FCPC column. It would be worth mentioning that the observed phenomenon may be of importance in the applications and designing of preparative and pilot-scale separations.

Identification of pure alkaloids obtained in the pH-zone-refining centrifugal partition chromatography was carried out on the basis of their ^{1}H, ^{13}C NMR and ESI-Mass spectroscopic data.

3.1. Chanoclavine

The compound isolated from pH-zone II in Fig. 2B showed positive ESI-MS, m/z 257 (M+H). The ^{1}H and ^{13}C NMR data of the alkaloid

(data not shown) matched with the reported NMR data for chanoclavine in literature [27].

3.2. Lysergol

The compound isolated from pH-zone III in Fig. 2B showed positive ESI-MS, m/z 255 (M+H). The ^{1}H and ^{13}C NMR data of the alkaloid (data not shown) matched with the reported NMR data for lysergol in literature [28].

4. Conclusion

The results of our studies showed that pH-zone-refining centrifugal partition chromatography produced efficient separations of two clavine alkaloids from gram quantities of *I. muricata* crude alkaloid extract. The present method may be successfully applied for the separation of various other alkaloids from natural products.

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