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UPDATED REVIEW ON EXTRACTION, ISOLATION AND QUANTITATIVE ESTIMATION OF ERGOT ALKALOIDS

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Ergot Alkaloids are potent α-blockers that cause direct smooth muscle contraction. They are products of fungi in the genus Claviceps. The most prominent member of this group is Claviceps purpurea (rye ergot fungus). Their common sources include Rye, bread as well as other grains. These are nitrogen derived- natural substances which are grouped under indole alkaloids. These ergot alkaloids are a diverse category of secondary metabolites that have been classified into 3 groups as Clavines, Amides of lysergic acid, Ergopeptines and Lactum ergot alkaloids based on their bond arrangement. They contaminate a large variety of cereals such as rye triticale, wheat and barley. In this present review, basic information regarding the ergots along with their chemistry, extraction, purification and isolation procedures for ergot alkaloids were focused, that helps to produce a purified crude extract that is taken for chromatographic analysis. This review also provides detailed informations on the chromatographic approaches for the ergot alkaloids estimations such as TLC, HPLC, HPTLC, UPLC /UHPLC, LCMS, GCMS with different detection techniques like Mass spectrometry and Fluorescence detection which was done in the past decade and also provides information regarding the pharmacological activity of ergot alkaloids like its application in the treatment of severe, throbbing headaches such as migraine, cluster headaches and also other therapeutic applications.

Keywords: *Ergot alkaloids, secondary metabolites, migraine, chromatographic approaches, Ultra Performance Liquid Chromatography.*

INTRODUCTION

Plant sources are ever advantageous for humans for their food for survival, energy and growth[1]. Herbals are majorly famous for TMS that relieves diseases. These are a pool of wide variety of active constituents which are rich in pharmacological activities. Amides and alkaloids are dominant secondary metabolites that were first spotted and utilized prehistorically i.e.4000 years before due to their pharmacological aptitude[2]. The word alkaloid was given by German pharmacist Wilhelm Meissner[3]. Pelletier defined Alkaloid as "A cyclic organic compound that contains nitrogen in a negative oxidation state". Alkaloids are identified in microorganism's marine organisms and mostly plants[4].Based on their molecular skeleton, Alkaloids are categorized into Indole alkaloids, Isoquinoline alkaloids, Tropane alkaloids, Steroidal alkaloids, Pyridine alkaloids,Pyrrolizidine alkaloids. According to Botanical origin, Alkaloids are again categorized into Opium alkaloids, Cinchona alkaloids, Rauvolfia alkaloids, Catharanthus alkaloids, Strychnous alkaloids, Cactus alkaloids,Solanumalkaloids[5]. Based upon Biogenesis, Alkaloids are categorized into True alkaloids(Alkaloids derived from Amino acids and contain nitrogen in Heterocyclic Ring.Ex: - Ergot alkaloids), Protoalkaloids (Alkaloids that have 'N' atom originatedfromAmino acids which does not include in Heterocyclic ring), Pseudo alkaloids (Alkaloids not originated from Aminoacids and have nitrogen in Heterocyclic ring[2].

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Ergot Alkaloids

Ergot Alkaloids (EA) are nitrogen-derived natural substances that are categorized under indole alkaloids[6]. Entire species that belong to Claviceps genus give rise to Ergot Alkaloids. The fungi producers belong to the phylum Ascomycota, e.g., Claviceps, Epichloe. Particularly, *Claviceps purpurea* over sclerotia originating upon rye, wheat additionally to other grains. Other crucial origins for alkaloids include Grasses tainted by entophytes (or) Claviceps species from fungi like penicillium and Aspergillus. As a whole ergot is defined as a fungus that infect various cereal plants and forms compact black masses of branching filaments that replace many of the grains of host plant[7]. Also, most natural Ergot Alkaloids have been separated and reported as the donator for ergotism as well as fescue toxicosis. The above-reported Ergot Alkaloids offersa foundation for the development of synthesis routes for Ergot alkaloids of pharmacological importance[8].

Chemistry based classification

The familiar composition or structure description of ergot alkaloids are given by Ergoline ring in Fig:1. Almost all Ergot alkaloids have double bond at C_8, $C_9$and C_{10}. The hydrogen atom at C_5 was mostly in β-configuration and the 'H' atom at C_{10} in 8-ergolenes has α-configuration. Carbon atom that was asymmetric at C8 of Ergolenes may produce epimers[9].

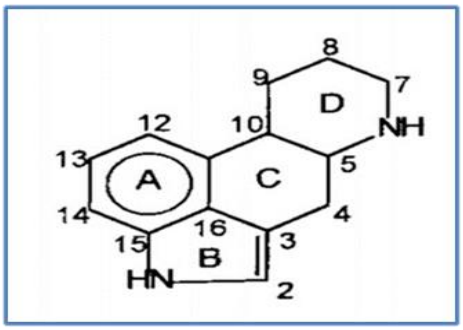

Fig. 1: Basic structure of Ergot alkaloids [9]

Based on R-substituent on C_8 that belongs to ergoline ring, Ergot alkaloids are differently categorized[7]. The categories include the following important groups: Lysergic acid alkaloids like Ergometrine; Clavine alkaloids that include hydroxyl and dihydroderivatives of 6,8-dimethyl-ergoline like Agroclavine; peptide alkaloids which include Ergopeptines, eg., Ergotamine, Ergovaline and Lactum Ergot alkaloids like Ergocristam[9-11].

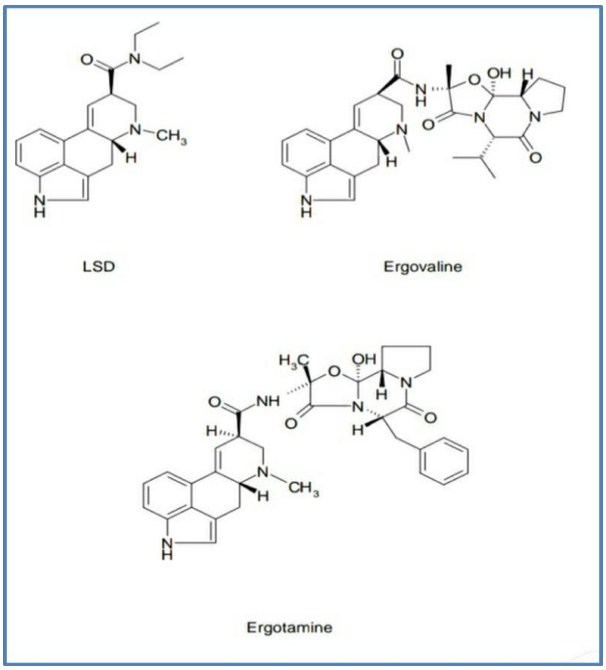

Fig. 2 : Structures of LSD, Ergovaline, Ergotamine [1]

The major step in sampling is the amount of substance required for analytical process [11]. No studies are conducted on the sampling procedures for grains or grain products for the analysis of ergot alkaloids. Yet, because of unequal distribution of ergots in the grains, the sampling preferred sample size is 1000-5000g for visual evaluation of ergots[12].

Extraction

Almost in all methods, which are used for reporting ergot alkaloids in cereals, extraction is obtained by non-polar organic solvents in basic conditions or by polar solvents in acidic conditions[12]. In modern chromatographic methods, simple mixtures of acetonitrile with ammonium hydroxide or ammonium carbonate were used [13]. Ergot alkaloids can be produced from the arid samples of different sources. The preferred extraction method for this is low polarity solvent mixtures by the inclusion of ammonium hydroxide which results in basic pH[11&13]. Best recoveries were seen for the mixture of acetonitrile and ammonium carbonate buffer in the 84:16 v: *v-ratio* when Ergot Alkaloids in cereals were evaluated for

extraction solvent. Recoveries ranges include from 91% - 121% indicating potent yields. Hence alkaline cases increase the Ergot alkaloids solubility[13]. Substitutional way of this is to make utilize polar solvents like Methanol, Acetonitrile in combination with dilute acids or buffers at low pH[11].

Generally, for TLC/Colorimetric Analysis, extraction is carried out by using solvents like chloroform, methanol and ammonium hydroxide. Then the extract was evaporated to dryness. The process is followed by partitioning of the residue obtained from ether solution by using acid continued by addition of ammonium hydroxide. Re-extraction was performed by using chloroform[7].

For modern analytical techniques like LC/MS, the extraction and filtration procedures are uncomplicated. Hence direct injection of samples into LC/MS can be done[13].

Incase of determining solitary ergot alkaloids in different sources, LC – Fluorescence detection can be used. In a mixed standard for use in Liquid Chromotographics, the concentration of ergopeptine alkaloids, ergotamine, ergoconine, α – ergocryptine, α – ergocristine was 10 µm/ml, ergonovine concentration include1 µm/ml[7].

For HPLC fluorescence detection, a weighed quantity of sample along with solvents and mixture of salts were taken in a 50 ml polypropylene tube and was shaken in a horizontal shaker for 30 min followed by centrifugation filtration and evaporation under the Nitrogen stream. Reconstitution was done with 0.4 ml of mobile phase and passed through 0.454 m of nylon syringe filter and subjected to HPLC-FLD[14].

In another HPLC- FD extraction process, the ergot alkaloids are extracted by drenching of *Claviceps sclerotia* in the solvent that is utilized for extraction for 60min followed by centrifugation, isolation and, purification. The resulting purified isolate is used for HPLC-FD[15].

In Agricultural products, EA is extracted by ELISA (Enzyme-linked immune sorbent assay). This is very simple and there is no requirement for cleanup. For example, the ergot alkaloids are extracted by a shaking methanol phosphate buffer solution that contains Tween-20 in 1:1, *v/v-ratio* with the sample or by also stirring sample with phosphate buffer saline solution[6].

For UHPLC-MS/MS analysis, samples of 500 g were ground in IKAM20 mill which results in the powder of particle size 0.5 mm. This milled sample was rinsed with ultrapure water and acetone followed by manual shaking in a large clean bucket for homogenization and then from the resulting test material 10 g were taken and incubated in 40 ml of acetonitrile and mixed properly with the help of ultra-turrax mixer for 3min and kept centrifugation for 10min at 4000g.The dilution ratio for 1ml of supernatant which is taken for instrumental analysis is in the 1:4 ratios with water[16].

For a Bio-analytical method, fundamental sections of tilters are gathered into liquid nitrogen and they are moved to freeze drier. Evaporated samples were ground and homogenized by using a Bead Ruptor in 7ml vial by making use of a ¼ inch Zirconium bead (30 s at 4.5 m/s). The samples were prepared by the extraction of 1mL of extracting solvent in 2ml plastic vials for 1hr by end over end rotation(30Hz) in the dark. Then they are kept for centrifugation 5000 x g for 5min and from the supernatant liquid, 600µl., was taken and diluted with 3.2 ml of Milli-Q water. These samples were taken for analysis after clean up[17].

In case of wheat milling products, 50ml, 84:16 (v/v) acetonitrile /3.03mM aqueous ammonium carbonate were used for extraction. The milled products were made into slurry and shaken in a Flatbed shaker for 30 min and comminuted in homogenizer for 3 min at 12000 rpm and then centrifuged. The supernatant was taken and diluted with 3.03Mm aqueous ammonium carbonate followed by internal standards addition to all samples before going into analysis[18].

Isolation and Purification

Purification is generally necessary for solvent extracts before analysis[13]. Hence, the clean-up can be done by Liquid-Liquid partitioning with the manipulation of acid/base effects of N-6 [12]. Solid- phase extraction occurs sometimes utilizing a strong cation exchange mode depending on acid-base separations. This SPE was taken into account for the biological fluids like urine or plasma[13]. For bio-analytical method, the samples which were prepared from

the extraction were taken onto previously conditioned and equilibrated Strata-X-CW SPE Cartridges by the process of centrifugation for 2min at 500 x g followed by subsequent washing of cartridges with water, 50% methanol and, eluted once with 5% formic acid in methanol and 1% ammonia in methanol. These final eluents were taken into the HPLC vials and made to dry by centrifugal evaporation then followed by resuspension with 200µL of 50% methanol and stored at -20^{0}C before analysis [17].The remaining cleanup process includes extrulent columns partitioning[12&13]. Partition of alkaloids between immiscible solvents, with the inclusion of salting-out methods, finding a polar solvent dissolution followed by an easy washing with hexane results in the sample that is suitable for LCMS/MS analysis[13]. The purification of crude extract was also done by preparative LC[19].

MATERIALS AND METHODS

Analytical Methods

Colorimetric analysis

The Colorimetric method for quantitative assay of ergot alkaloid was performed in liquid culture filters with the addition of van urk reagent (0.125% p-dimethylamino benzaldehyde in 63% sulphuric acid+ 0.1% of 5% ferric chloride) by using lysergic acid as standard[20]. A positive reaction is the appearance of blue color at 580nm. These colorimetric methods are utilized for grain ergot extracts and artificially contaminated triticale grain at 35% of ergot.The composition in van-urk reagent is altered by Michelon and Kelleher where they substituted ferric chloride with sodium Nitrate which was reported with better sensitivity and stability[7].

TLC

It is not possible to report the ergot alkaloids contents by visual detection. Hence we need the help of analytical techniques[7]. Previously, paper chromatography was used sooner a TLC came into existence as they are inexpensive and relatively rapid. TLC methods can isolate isomers and have a low limit of detections. According to a survey, the drawbacks of TLC are specificity is less and it interferes with the other components[11]. Generally in TLC, the stationary phase used mainly is silica gel and its substitution is alumina. The solvent system includes chloroform, Benzene, ethanol, acetic acid in their combinations. Then they are checked for fluorescence under UV[7, 11, 12&21].

HPLC

RP-HPLC plays a prominent role in the estimation of ergot alkaloids. The first reported HPLC analysis of ergot in combination with LSD is by Jane and Wheals[8]. HPLC-FD is used in-case of individual ergot alkaloids should be quantified[13]. The solvent system includes chloroform, diethyl ether, isopropanol and ethanol[22]. Other solvents include acetonitrile and injection volume was 100ml[13]. For detection purpose, fluorescence detection isused.Ergometrine, ergotamine, ergoconine, ergocryptine, ergocristine, ergosine are the ergot alkaloids that are usually analyzed by HPLC. Total alkaloids content is the sum of all ergot alkaloids [23].

UPLC/UHPLC

Ergot alkaloids are estimated by Acquity UPLC in combination with MS [16]. Solvent systemincludes Ammonium carbonate, Acetonitrile (10:3); Water and Acetonitrile with 0.1% v/v Formic acid [16&24]. Flow rate include 0.4m/min [16]. Nitrogen can be taken for desolvation. The column used is C18 (2.1×100 mn) with 1.74µm particles. These UPLC are connected to MS which functions prominent role[25].

LCMS

LC coupled to MS and LC Tandem MS generally utilize ESI in a positive mode for estimation of ergot alkaloids[12]. Flow rate include 0.2 ml/min. Column details are 150 mm×20 m.Mobile phases include 0.1%v/v aqueous formic acid and 0.1% formic acid in acetonitrile. Run time is 43 min[26].

GC/MS

As Ergot alkaloid decomposes in the hot injector, GC is not preferable but the peptide portions can be reported by GC/MS[23]. Electro impact, GC/MS can measure LSD and Iso LSD in urine samples. The recoveries till now are up to 69% and hence these are not mostly used[13].

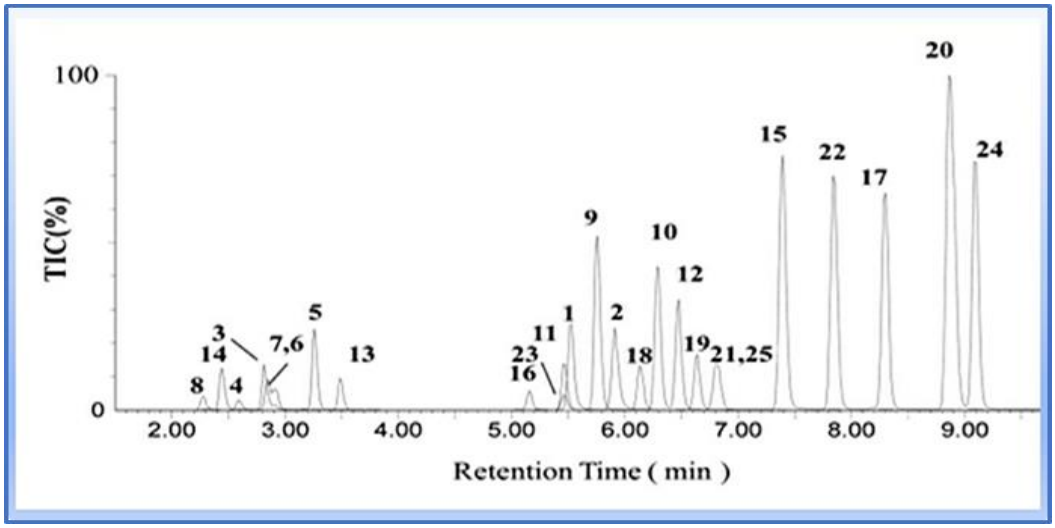

Fig 3: Estimation of Ergot alkaloids by UPLC[2]

Table 1: Estimation of ergot alkaloids by different Liquid Chromatographic Techniques

S.no	Compound	Source	Analysis	Detector	Mobile Phase	Chromatographic conditions	Ref
1	Ergot alkaloids	Epichloefestucae	HPLC	MS – ESI	Ammonium carbonate Acetonitrile	C_{18} column	[17]
2	Ergot alkaloids	Rye , wheat	UHPLC	Tandem mass spectrometry	Acetonitrile, Ammonium carbonate	BEH C_{18} column	[28]
3	Ergot alkaloids	Swine feed	UHPLC	Tandem mass spectrometry	Acetonitrile, Ammonium carbonate	BEH C_{18} column 0.4mL/min	[29]
4	Ergot alkaloids	Oats based functional foods	HPLC	MS	Methanol & Water with 0.1% Formic acid	Zorbax eclipse plus C_{18} column 0.4mL /min	[30]
5	Ergot alkaloids	Cereal samples from Algeria	UHPLC	MS	Acetonitrile, Ammonium carbonate	C_{18} column 0.4mL /min	[31]
6	LSD	Metarhizium species	HPLC	FLD	Methanol	C_{18} column	[32]
7	Ergot alkaloids	Breads in Netherlands	UPLC	Tandem Mass Spectrometry – ESI	Acetonitrile, Ammonium carbonate	BEH C_{18} column 0.4mL/min	[33]
8	Ergot alkaloids	Wheat, Triticale, Rye, Fodder pellets	UHPLC	MS	Acetonitrile & Water with 0.1 % Formic acid	BEH C_{18} column 0.4mL/min	[16]
9	Ergot alkaloids	Plectenchym-atic Mycelia	TLC	Colorimetric analysis	Ethyl acetate, Ethanol, Diethyl formamide	TLC Plates Silica gel – G	[20]
10	Ergot alkaloid	Rye	HPLC	ELISA	Acetonitrile, Ammonium carbonate	C_{18} column	[34]
11	Ergot alkaloid	Cereal grain intended for animal feeding	UPLC	MS	Acetonitrile, Ammonium carbonate	Phenyl Hexyl Luna column	[35]
12	Mycotoxins	Dairy cattle & poultry feed ingredients	LC	MS	Methanol, Water, Acetic acid, Ammonium acetate	C_{18} column 1000µL/min	[36]

Table 1: Continued

S.no	Compound	Source	Analysis	Detector	Mobile Phase	Chromatographic conditions	Ref
13	Ergot alkaloids	Wheat & Rye derived products in Italy	UHPLC	MS with Triple quadrapole	Ammonium carbonate, Acetonitrile	BEH C_{18} column 0.4mL/min CT: 40^0C	[37]
14	Ergot alkaloids	Pasta	HPLC	ESI- Positive mode	Ammonium carbonate, Acetonitrile	C_{18} column 1mL/min	[18]
15	Ergot alkaloids	Rye products	LC	FLD	Aq.Ammonium carbonate, Acetonitrile	Phenomonex Luna phenyl hexyl column	[38]
16	Ergot alkaloids	Phyllantus niruri Linn.	TLC	Densitometry	Chloroform, Methanol, Ethyl acetate, Ammonium hydroxide	TLC Plates	[39]
17	Ergot alkaloids	Cereals from Luxembourg	UHPLC	FLD	Acetonitrile, Water	BEH C_{18} column 0.2 mL /min	[40]
18	Ergot alkaloids & Mycotoxins	Wheat & Maize	UPLC	MS-ESI	Aq. 0.3% Formic acid with Ammonium formate	BEH C_{18} column 0.4 mL /min CT:- 30^0C	[41]
19	Ergot alkaloids	Maize	HPLC	MS	Acetonitrile, Water, Formic acid	C_{18} column 500µL/min	[42]
20	Ergot alkaloids	Western Canadian grains	HPLC	Tandem mass spectrometry	Acetonitrile, Ammonium acetate	C_{18} column	[43]
21	Ergot alkaloids	Rye flour	HPTLC	FLD	Methanol, water	6.0 x 3.0mm plates	[44]
22	Ergot alkaloids	Rye containing breads	HPLC	MS, FLD	Methanol, Water	C_{18} column	[45]
23	Lysergic acid amide	Morning glory seeds (*Ipomea violacea*)	GC	MS	Chloroform, Methanol, Ammonium hydroxide	C_{18} column FR –1ml/ min	[46]
24	Ergot alkaloids	*N.fumigata*	HPLC	FLD	Acetonitrile, Ammonium acetate	C_{18} column	[47]
25	Mycotoxins	Rye, Barley	HPLC	MS	Methanol, Water, 0.1% Formic acid	C_{18} AQ column 350µL/min	[48]
26	Ergot alkaloid	Wheat from Albania	LC	MS	Acetonitrile, Ammonium carbonate	Phenyl hexyl column	[49]
27	Ergot alkaloid / Mycotoxins	Animal feed maize samples	LC	MS	Methanol, Water, Ammonium acetate	C_{18} column	[50]
28	Ergot alkaloid	Cereal samples	UPLC	MS	Acetonitrile, Ammonium carbonate	ACQUITY BEH C_{18} column	[25]
29	Ergot alkaloid	Rye flour	HPTLC	MS – ESI	Methanol, 0.1 % Formic acid	20 x 10cm plate 200µL/min	[51]
30	25 Ergots	Cereal samples	UPLC	MS	Acetonitrile, Ammonium carbonate	BEH C_{18} column 0.2 mL/min	[27]
31	Ergot alkaloid	Animal feeding stuff	HPLC	FLD	Acetonitrile, Ammonium carbonate	Luna C_{18} column 0.7–1mL /min	[14]
32	Ergot alkaloid	Cereals	HPLC	MS	Acetonitrile, Ammonium carbonate	C_{18} column	[23]
33	Ergot alkaloid	Sclerotia of *Claviceps purpurea*	HPLC	MS – ESI	Methanol, Water, 1 % Formic acid	Phenyl hexyl column 250µL/min	[52]

Table 1: Continued

S.no	Compound	Source	Analysis	Detector	Mobile Phase	Chromatographic conditions	Ref
34	Ergot alkaloid	Grain products	LC	MS – ESI	Acetonitrile, Ammonium carbonate	Sphinx RP 1.8µm column	[53]
35	Ergot alkaloid	Cereals	UHPLC	HRMS	Acetonitrile, Methanol	Hypersil gold column 400 µL/min	[54]
36	Ergot alkaloid	Epichloe	HPLC	FLD	Acetonitrile, Ammonium carbonate	C_{18} column 0.9mL/min	[55]
37	Fumiga clavines D-H	Entophytic *Aspergillus fumigates*	RP-HPLC	NMR, FTIR, UV	Petroleum Ether, Acetone	C_{18} column	[56]
38	Ergot alkaloid	Tall fescue seeds	HPLC	FLD	80% Methane, Ammonium acetate	C_{18} column	[57]
39	Ergot alkaloid	Cereals	HPLC	MS-ESI	Methanol, Water, Ammonium carbonate	C_{18} column 0.15mL/min	[58]
40	Ergot alkaloid	Barley	HPLC	MS	Ammonium bicarbonate, Methanol, Water	X Bridge C_{18} column	[59]
41	Ergot alkaloid	Cereal & its products	HPLC	MS – ESI	Methanol, Ammonium carbonate, Acetonitrile, Water	X Bridge C_{18} column	[60]

Therapeutic Applications

Ergot Alkaloids functions as potent drugs because of their well-built interplay with dopamine, serotonin and adrenergic receptors of CNS, also adrenergic receptors in blood vessels[61].

Ergot alkaloids play a major part in the therapy of migraine. Two drugs ergotamine and dehydro ergotamine are taken for treating migraine and these two drugs vary in pharmacokinetic and pharmocodynamic features. Caffeine content in these drugs help them to absorb faster into the body specifically Dihydro-ergotamine is being the major choice to treat migraine headaches. Hence they are called "Migraine specific drugs[62].

Ergot Alkaloids plays a major role in cancer therapy. In prostate cancer, chemo resistance is the major hindrance. Ergot alkaloids such as dehydroergocristine was approved to treat this chemo resistant prostate cancer. The effects of the drug were reported by quantitative PCR, Western blot analysis and reporter assay[63].

These EA are also applied in ocular pharmacology. They are also used in the treatment of glaucoma[64].These EA also seems to produce vasoactive effects based on the in-vitro studies conducted[65]. EA and their derivatives has been traditionally utilized in blood pressure regulation and also aids in child birth[66].

Bromocriptine, which is a dopamine D2 receptor agonist, has been widely used for patients with Parkinson's disease[67].Stimulatory effect has also been observed on the motor activity of uterus by various Ergot alkaloids[62].

Conclusion

The study provides informations on sample preparation techniques on different analytical methods that are possible for estimation, determining and reporting of Ergot alkaloids from different sources such as *Claviceps purpurea*, cereals bread, etc along with extraction, isolation and purification procedures. The techniques focused so far include TLC, HPLC, HPTLC, UPLC, LCMS with MS, FLD detection procedures. In limited cases, Colorimetry and ELISA also provides information which is limited and hence they are used as a semi quantitative tool. According to study, the UPLC method for the estimation of alkaloids provides better results which include low solvent consumption and less analysis time.

Abbreviations

- **EA –**Ergot Alkaloids
- **LSD –** Lysergic Acid Diethylamide
- **TLC –** Thin Layer Chromatography
- **HPLC –** High Performance Liquid Chromatography
- **HPTLC –** High Performance Thin Layer Chromatography
- **UHPLC –** Ultra High Performance Liquid Chromatography
- **MS –** Mass Spectrometry
- **FLD –** Fluorescence Detection
- **ESI –** Electron Spray Ionization
- **HRMS –** High Resolution Mass Spectrometry
- **ELISA –** Enzyme Linked Immuno Sorbent Assay
- **PCR –** Polymerase Chain Reaction
- **TMS –** Traditional Medicine System

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مرجع محدث للاستخراج والفصل والتقدير الكمي لـــقلويدات الإرجوت

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قلويدات الإرجوت هي ألفا بلوكر قوية تسبب تقلصًا مباشرًا للعضلات الملســاء. إنهــا نتــاج فطريات من جنس كلافيسبس. و الأبرز في هذه المجموعة هو كلافيسبس بوربيوريا (فطــر إرجــوت الذرة). تشمل مصادرها الذرة و القمح بالإضافة إلى الحبوب الأخرى. هذه تالقلويدات عبارة عن مــواد مشتقة من النيتروجين – مواد طبيعية تندرج تحت قلويدات إندول. قلويدات الإرجوت هــذه هــي فئــة متنوعة من المستقلبات الثانوية التي تم تصنيفها إلى ثلاث مجموعات مثل كلافينات ، أميــدات حمــض الليزرجك ، أرجوبيبتينات ، لاكتام قلويدات الإرغوت بناءً على ترتيب الروابط الخاصة بهــم. أنهــا تلوث مجموعة كبيرة ومتنوعة من الحبوب مثل الذرة والقمح والشعير.

في هذا التقرير ، تم التركيز على المعلومات الأساسية المتعلقة بالإرجوت إلى جانب الخــواص الكيميائية و الاستخلاص و الفصل والتنقية لقلويدات الإرجوت ، مما يساعد على إنتاج مستخلص خــام منقى يتم أخذه للتحليل الكروماتوجرافي. يوفر هذا التقرير أيضًا معلومــات مفصــلة عــن الأســاليب الكروماتوجرافية لتقديرات قلويدات الإرجوت مثل كروماتوجرافيا الطبقة الرقيقة و استشراب الســائِل الرفيع الإنجاز و كروماتوجرافيا الطبقة الرقيقة رفيع الانجاز و كروماتوجرافيا السائِل مع مطيــاف الكتلة و كروماتوجرافيا السائِل مع مطياف الكتلة مع تقنيات الكشف المختلفة مثــل قيــاس الطيــف الكتلي واكتشاف التألق الذي تم إجراؤه في العقد الماضي ويوفر أيضًا معلومات تتعلق بالنشاط العلاجي لقلويدات الإرجوت مثل تطبيقها في علاج الصداع الشديد النابض مثل الصــداع النصــفي والصــداع العنقودي وأيضًا التطبيقات العلاجية الأخرى.