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Analytical Method Determination Of Betulinic Acid:A Overview

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ABSTRACT: Betulinic acid is the pentacyclic triterpenoids which occurred naturally then exhibits the potent anticancer activity.Thisreviewaims to compiling the published analytical method for the determination of the Betulinic acid. By using the techniques like UV visible spectrophotometry, HighPerformanceLiquidChromatography(HPLC),High Performance Thin Layer Chromatography (HPTLC) Tandem MassSpectrometry, LC/MS/MS, Ultra PerformanceLiquid Chromatography (UPLC),Thin Layer Chromatography (TLC). Further method validation is carried out to ensure the method developed was accurate,specific, reproducible,precise over the specified range in which an analyte is analysed.

KEYWORDS: Betulinic Acid, UV, HPLC, HPTLC, LC/MS/MS, GC, TLC.

I. INTRODUCTION

Betulinic acid is pentacyclic triterpenoids discovered in 1995 in the stem bark Melaleuca cajuput showing the cytotoxicity towards a number of cancer cell line. Betulinic acid found in bark of many plants and it is an oxidation product of low molecular compound called Betulin a substance found in the outer bark of White Brich bark treeBetula alba. It acts as anticancer agent. It also used as antiproliferative, antinflammatory, anti-HIV, antimicrobial, antiretroviral,[2] but it naturally exhibits the potent anticancer activity.[1,3,]Betulinic acid pure compound appears as White crystalline solid that melts at 295ºC- 297ºC. Betulinic acid was highly soluble in pyridine and acetic acid. The solubility of organic alcohol such as Methanol, Ethanol, Chloroform, Ether is reduced. Petroleum ether, Dimethyl formamide, Dimethyl sulfoxide, Benzene has low solubility.[3]

1.1. CHEMISTRY

It is pentacyclic lupine type triterpene. It is 3β -hydroxy-lup-20(29)-en-28-oic acid. IUPAC Name is1R, 3as,5ar,5br,7ar,9s,11ar,11br,11br,13ar,13br)-9hydroxy -5a,5b,8,8,11a-pentamethyl-1prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chr.

Its chemical formula $C_{30}H_{48}O_3$.Molecular weight is 456.7g/mol. It is C-28 carboxylic acid derivative of triterpene betulin.[4]Structure of Betulinic acid given in fig no .1.

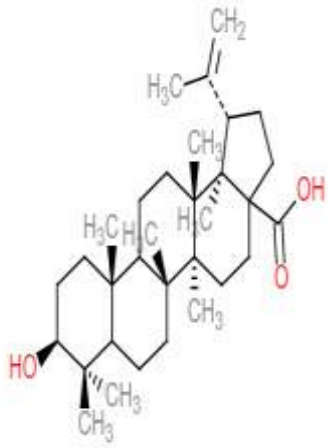

Figure no.1 – Structure of Betulinic Acid

1.2.Mechanism of Action of Betulinic Acid: -

Betulinic acid having potent anticancer activity. Its ability to induce apoptotic cell death in cancer cell by triggering the mitochondrial pathway of apoptosis. Apoptosis is an intrinsic program of cell death that is present in every cell and regulated by defined signalling pathway.

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The main mechanism of anticancer action of Betulinic acid is known as the induction of apoptosis in cells,Betulinic acid induced the death cell which occurs due to cancer cell. That death of the of the normal cell is controlled by the Betulinic acid.The pathway for induction of the Betulinic acid is mitochondrial Pathway. The tumor suppression protein involved in apoptosis is the P53 (cellular tumour protein antigen).But the apotosis process is independent on the P53. The apoptosis in Mitochondrial membrane permeation in which the release of the cytochrome -C from the mitochondria andcytochrome-C dependent formation of a capase-3 activation complex. caspase -3 activation complex induces the death receptor and activates the pathway by which kill the cell in mitochondrial membrane permeation. Capacase also act in DNA fragmentation of cancer cell.[3,4,5,6,7]

1.3 Pharmacology –

A) Pharmacokinetics –

The pharmacokinetics and tissue distribution of Betulinic acid was studied in CD 1(cluster of differentiation 1)mice afteri.p 250 and 500mg/kg dose the serum concentration reached peak at 0.15 and 0.23 hr. The elimination halfliveswas 11.5 hr and 11.8 hr. Total clearance of 13.6 and 13.5 L/kg/h. [3]

B)Side effect –

Betulinic acid is anticancer drug but it having less side effect. such as diarrhoea, anorexia.

II. SAMPLE PREPARATION SATERGIES –

Sample preparation is integral part of analytical methodology and it was reported that approximately 30% error is contributed from sample analysis was due to sample preparation. The various diluents like Acetonitrile, Methanol, Water, Acetone,Ethanol. In major cases Acetonitrile and Methanol used as diluent. The sample preparation technique for the extraction of Betulinic acid by using Ethyl acetate, Hexane,Toluene, Formic acid.

III. ANALYTICAL METHOD-

This all methods are used for determination of Betulinic acid. This allanalytical method is reported during the literature survey. All reported analytical method are at specific condition. The literature survey gives the number of methods for determination of betulinic acid**.**

3.1Spectrophotometry:

In the literature survey were found that UV spectrophotometric method have been reported for determination of Betulinic acid. In that the simultaneous method, derivative method, Q-absorption method, Area Under Curve etc. all are the method of UV spectroscopy for the determination of Betulinic acid but the simultaneous method was majorly used for the determination of Betulinic acid.[8,9]

Table no 1- showed the summery of reported UV - spectrometric methods indicating sample matrix used, λ^{max}, solvent used in it.

Sr no	Name of drug	Method	Wavelength	Solvent	Ref no
1	BETULINIC ACID	Simultaneous method	210nm	Ethanol	1
2	BETULINIC ACID	-	210nm	Ethanol	10

Table no 1: Summary of UV spectrometric method of Betulinic acid

3.2 Chromatographic method:

TheHighPerformanceLiquid Chromatography for determination of Betulinic acid. Table no 2. Shows the summarized reported chromatographic method indicating sample, method, mobile phase composition, wavelength, Detector, LOQ, LOD.

According to the literature survey of the Betulinic acid byHPLC method, there are different method for determination of Betulinic acid such as Reverse Phase High Performance Liquid Chromatography, Isocratic High Performance Liquid Chromatography, Gradient High Performance Liquid Chromatography, Normal phase High Performance Chromatography etc. but the Reverse Phase High performance liquid Chromatography was majorly used.

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Table no. 2- summarized method for determination of the Betulinic acid by using HPLC.

Sr no	Drug	Combination	Mobile phase	Column	Detector	Flow rate ml/min	λ max (nm)	LOD µg/ml	LOQ µg/ml	Ref no
1	Betulinic acid		Methanol: acetonitrile: water (90:5:5v/v/v)	RP C18 column	UV	1.3	270	0.144	0.436	12
2	Betulinic acid	Urosolic acid	Acetonitrile: methanol (80:20)	C 18 column	UV, MS	0.5	210	-	-	11
3	Betulinic acid	Betulin	Acetonitrile: Water (86:14 v/v)	Diamonsil C18 Reversed phase column	UV	1	210	0.31	-	1
4	Betulinic acid		Acetonitrile: Methanol: Acetic acid acidified acid water by pH 2.8 (70:20:10 v/v/v)	Reversed phase C18 column (Hibar Rt 250×4mm i.d, Lichrosrob RP 18, 10 µm)	UV	1	210	0.0005	0.0050	13
5	Betulinic acid		Acetonitrile: Methanol (80:20)	C18 (4.5×250 mm, 5µm)	UV	0.5	210	-	-	14
6	Betulinic acid		Acetonitrile: Water (15:15 v/v)	Reverse phase Hypersil C18 column ,250mm	PDA	1	210	8.24 ppm	24.73 ppm	15
7	Betulinic acid	Urosolic acid	Acetonitrile: Water (90: 10 v/v)	Reversed phase C18 column	UV	1	210	0.268	0.878	16
8	Betulinic acid		Acetonitrile: Water (92:08 v/v)	C18 column (250 mm×4.6 mm, 5mm	DAD	1	205	0.1	0.3	17

3.4- HPTLC

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HPTLC method for determination of Betulinic acid. Table no 3- shows the summarized reported HPTLCmethod indicating sample, wavelength, mobile phasecomposition,linearity, retention factor.

Table No 3- Summary HPTLC Method of Betulinic acid.

Sr.no	Drug name	Combination	Mobile phase	Linearity	ʎ max	RF	Ref .no
1	Betulinic acid	-	Hexane: ethyl acetate (6:4 v/ v)	0.2-500µg	366nm	-	18
2	Betulinic acid	-	Toluene: ethylacetate: methanol (16:2:2 v/v)	0.99 µg	540nm	0.67	10
3	Betulinic acid	-	Hexane: Ethyl acetate: Formic acid (3:2:0.02 v/v/v)	0.005-100 µg/ml	366nm	-	13
4	Betulinic acid	UA, BA, LUP, STGM.	PET ET: ethyl acetate: toluene (7:2:1v/v/v)	100-600ng/ml	540 nm	UA-O.21 BA-0.29 LUP-0.50 STGM-0.33	19
5	Betulinic acid	-	Toluene: Methanol: Formic acid (8:1:1v/v/v)	0.2-1.2 µg	540 Nm	0.47	20

3.5 –UPLC(UltraPerformance Liquid Chromatography)-

Betulinic acid was determined in the extract of Disporosis pernyi diels by Ultra Performance Liquid Chromatography. UPLC analysis was done by using Shim Pack XR-DDS column with gradient elution.mobile phase was Acetonitrile: water: formic acid. Flow rate was 0.2 ml/min. Detection of Betulinic acid at 210 nm. Linearity was found to be 48.34- 338.10 µg/ml. Regression coefficient was found to be 0.999. LOD was found to be o.137µg/ ml.LOQ was found to be 0.456 µg/ml.[21]

3.6 -LC-MS (LiquidChromatography – Tandem Mass Spectrometry) method-

Development and validation of Betulinic acid in rat plasma by using Liquid chromatography -Tandem mass spectrometry method. An agela MG- C18 analytical column (50×2.1 mm 5 µm). mobile phase was methanol: water: formic acid (80:20:0.1 v/v/v). flow rate is 0.6ml/min. Mass detection was conducted by electrospray ionization. Milli Q ultrapure water purification system used for deionized water used in LC-MS-MS. Linearity was 3 ng/ml. R^2 was 0.9961-0.9976 . Interday precision<7.8, Intraday precision>5.7. [22]

Simultaneous quantification of Betulinic acid madhucalongifolia methanolic extract of bark by using LC-MS. Stationary phase Gemini C18 column (50mm ×2mm, 3 µm). mobile phase acetonitrile:methanol (50:50v/v). run time 5min. Flow rate was 0.4 ml/min. Retention time for Betulinic acid was 1.25.[23]

Quantitative analysis of Betulinic acid in rat plasma by using LC- MS,for analysis isocratic reversed phase system coupled to negative ion electrospray mass spectrometer, Agilent G19 G6A LCMSD quadrupole mass spectrometer equipped with a series 1100 HPLC binary pump. C18 column [5 µm: 250 ×2.1mm]. 90% acetonitrile: water used as mobile phase. Flow rate 0.2 ml/min. LOD was found to be 0.5pg. LOQ was found to be 2 pg.Intradayprecision was found to be 9%, Inter day

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precision 5.9 %. regression coefficient (r^2) was found to be 0.999.(24)

Determination of Betulinic acid by using LC-MS method, using positive electron spray ionization,isocratic, column C18 RP (150 mm×4.6mm 5µm). 80% acetonitrile: ammonium acetate. flow rate 1ml/min. LOD was 0.087 µg/ml. LOQ was 0.266 µg/ml. Repeatability was 1.6%. intermediate precision was 2.07 %.(25)

3.7 GC/MS(Gas chromatography – mass spectroscopy) method-

Analysis of the presence of Betulinic acid in the leaves of Eugenia florida by Gas chromatography-mass spectroscopy, GC/FID. GC performed by 6890N, HP 5 column (50×0.25mm, 0.25µm, liquid phase). Oven temperature was maintained 70ºc-300ºc at 5ºc\min. carrier gas was helium 11 3 L/min. split mode 20:1. R^2 was found to be 0.9994. Linearity for GC/FID was found to be 0.1- 0.5 µg/ml. (26)

Abbreviation-UV-UV-Visiblespectroscopy, HPLC- High Performance Liquid Chromatography**,** HPTLC- High Performance Thin Layer Chromatography, UPLC- Ultra Performance Liquid Chromatography, LC- Liquid Chromatography, GC- Gas Chromatography, MS- Mass Spectroscopy, LOD-Limit of Detection, LOQ-Limit of Quantification, UA- Urosolic acid, LUP-Lupeol, PET-ET- Petroleum Ether, STGM – Stigmasterol,

IV. DISCUSSION-

The different analytical method are used for the determination of the Betulinic acid. The presented review helps to decide which mobile phase, stationary phase, instrument was gives the better analysis of Betulinic acid. Review gives the different technique so analyst can select the proper technique for the determination of Betulinic acid. The effective combination parameter should minimize the cost of the analysis, reduce the time by using the selective analytical method.

V. CONCLUSION-

The Review article presented the analytical method for determination of Betulinic acid. The literature survey of analytical data exhibits that HPLC methods are primarily for the analysis of Betulinic acid. Determination of Betulinic acid is also done by the HPTLC, GC-MS, UV, LC-MS method, concluded the method developed was accurate, specific, precise, reproducible.The presented information is useful for the future prospective study for researcher in analysis of Betulinic acid.

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CONFILCT OF INTREST –

The author shows that there is no conflict of intrest.

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