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SELECTION OF RESIN FOR STEVIOL GLYCOSIDE EXTRACTION FROM STEVIA LEAF

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Abstract: The Food industry has shown tremendous interest in alternative sweeteners to sugars to manufacture healthier products. Monk Fruit is a rich source of low-calorie sweeteners. But in the extraction process, the selection of Adsorbent resin is a major issue, so a set of experiments were conducted to overcome this issue. And stevia leaf and hot water(50-55°C) were taken in the ratio of 1:5.and then flocculation was done with calcium hydroxide. After that, filtered and at the adsorption step, different adsorbent resins were used. There were four resin XAD 4,7,16 & 1180 on different BV 1,2 & 3.for desorption different concentration 40%,60% & 100%. After the above experiments on XAD 16 with 2 BV and 40%, Elution has shown the highest yield. After that, it can further process for extraction of steviol glycosides.

Keywords **- Steviol Glycoside, low calorie sweetener, Elution & BV(Bed Volume)**

I. INTRODUCTION

The utilization of starches, including sugar use, is acquiring expanded consideration because of stoutness and diabetes (KEARNEY, 2010). The expanded accessibility of sugar has worked with a rising number of energy-rich, unhealthy groceries available. This significantly affects those attempting to lead a sound way of life. Subsequently, there is a requirement for low-calorie regular sugars, which can supplant sucrose . Today, numerous artificial sugars exist, for example aspartame, saccharin, sucralose, cyclamate, and so forth, which assume no part as far as energy supply, just concerning improving. In any case, these sugars can't match the flavor of genuine sugar, and during the utilization of these fixings clinical secondary effects can emerge. Hence, the objective is to make an elective sugar, which agrees with every one of these requirements. On the other hand, there are a few normal low-calorie sugars tracked down in nature that, when consumed, are equipped for staying away from the previously mentioned secondary effects. The vast majority of them are separated from plants, and their sweet flavour has been utilized in certain societies since antiquated times, for example agave nectar, priest organic product, maple syrup, and so on, however the modern extraction of them isn't normal, since sugar is modest and effectively available.The plant Stevia rebaudiana fills normally in South America. Substances created by extraction from stevia are multiple times better than sucrose. Stevia has been utilized for quite a long time by the native Guarani clan as a sugar and a home grown tea to treat indigestion and different grievances. The plant has a place with the Asteraceae family. In excess of 230 species have a place with the family Stevia, however just the species phlebophylla and rebaudiana produce parts with sweet taste.

The sweet taste is brought about by the gathering of diterpene glycosides. Moreover, close to its sweet parts, the plant contains various other significant mixtures, for example tannins, fl avonoids, alkaloids, lipids, nutrients, minerals, and medicinal oils .The plant contains eight unique steviol glycosides: stevioside, steviolbioside, rebaudioside-A, - B, - C, - D, - E, and dulcoside A. The most widely recognized is stevioside, which contains 4-13% of the plant dry matter and rebaudioside A, which can be viewed as in 2-4%. These are trailed by rebaudioside-C (1-2%) and dulcoside A (0.4-0.7%). Rebaudioside-B, - D, - E, - F, and steviolbioside are available in the plant as minor parts. These particles are exceptionally steady in watery arrangements over a wide pH as well as temperature range. They are not straightforwardly fermentable. Steviosides are separated by gastrointestinal bacterial fl ora into aglycone (for example steviol) and sugar, however the sugar isn't caught up in the colon, so steviol glycosides have a zero-energy content .Because of the benefi cial impacts referenced over, various examinations have proactively understood the extraction of steviol glycosides from the stevia plant species . These incorporate conventional and forward-thinking strategies.Stevia rebaudiana is a plant that starts from Paraguay, South America, and it delivers high strengthlow-calorie sugars in its leaves, principally stevioside and rebaudioside A, both steviol glycosides. Locally,the plant leaves have been utilized for their improving limit since some time in the past, yet not until the 1960's wasbusiness development began in Paraguay and Japan, and later in different nations too. In the last part of the 1990'sa large portion of the Stevia development was occurring in China, with Japan being the significant market. Stevioside and rebaudioside A separated from Stevia leaves are currently pretty much generally utilized in Japan, South Korea, China,South-East Asia and South America, as a sugar in a wide assortment of food sources. Since the endorsement of Stevia sugars in the US by the FDA in 2008, and by the European Union in 2011, modern interest has risen as needs be.For the extraction and refinement of the steviol glycosides from the plant material, a few prospects exist. A usually utilized extraction strategy comprises of separating dried and powdered leaves with high temp water,after which an essential explanation is reached by filtration

and centrifugation. One more typical technique for the extraction of leaves utilizes an ethanol-water blend, trailed by a dissipation of the concentrate. Other procedures incorporate explanation utilizing hexane, or dissolvable extraction followed by decontamination utilizing specific adsorption by particle trade, or expansion of chelating specialists followed by crystallization, or extraction followed by adsorption utilizing zeolites . For purging purposes, ultra-and nanofiltration are likewise proposed, including a centrifugation step for explanation of the concentrate, in a review utilizing dried and powdered Stevia leaves .To diminish process costs connected with drying, it could be desirable over process new Stevia, perhaps at somewhat limited scope - for example near the area of development. In two examinations performed already to the one portrayed in this report , new Stevia plant material is separated in water at room temperature, as proposedby the organization Newfoss . To work with the extraction of steviol glycosides through the cell wall, the water is fermented to advance cell wall porousness;

Stevia is a "wonder crop" with a solid sweet taste that has been utilized to improve beverages and make tea since the sixteenth 100 years. During World War II, England began to inspect stevia as a substitute for sugar, which was elusive. However, during the 1970s, the Japanese began to utilize stevia to supplant the restricted counterfeit sugar, Saccharin. Right now, the stevia plant is filled in different countries all over the planet, like India, China, Vietnam, Brazil, South America, South Korea, Taiwan, Israel, Argentina, Colombia, and so on.Stevia is a "**wonder crop**" with a strong sweet taste that has been used to sweeten drinks and make tea since the sixteenth century. During World War II, England started to examine stevia as a substitute for sugar, which was hard to find. But in the 1970s, the Japanese started to use stevia to replace the banned artificial sweetener, Saccharin. Currently, the stevia plant is grown in various nations around the world, such as India, China, Vietnam, Brazil, South America, South Korea, Taiwan, Israel, Argentina, Colombia, etc. In 1971 Japan started to use stevia in its food. In 1984 China started its cultivation, in 1991 stevia was banned in the United States because of the early investigations that recommended the sweetener may cause cancer. However, in December 2008, the United States Food and Drug Administration(USFDA) acknowledged this contention, announced stevia GRAS and permitted its use in standard U.S. food production. In 2011 European Food Safety Authority approved the use of Steviol Glycosides as a sweetener and finally in 2015 FSSAI of India allowed the use of stevia in food items and beverages. Stevia cultivation typically requires around 20 percent of the land and far less water to give a similar measure of sweetness as other standard sugars. Stevia farming gives a productive harvest to thousands of independent farmers having small lands. It does not replace food crops but is being cultivated as a cash crop on smaller plots in addition to food crops for an increase in revenue. When growing conditions are most favorable, farmers may harvest stevia several times per year. Stevia is otherwise called Sweetleaf, Honey leaf, and Sugar leaf. However its cultivating is finished all through the world for quite a while, it has been around twenty years when the development of stevia plant was presented in India and as of now it is experiencing childhood in an extraordinary manner. At present, India has around 30 million diabetic patients, as most would consider to be normal to increment to 80 million until 2025. Along these lines, the Indian ranchers have additionally begun to take stevia development to the powerful following the gigantic interest for the diabetic market here. As of now India's complete yearly creation of stevia is around 600 tons. The atmospheric conditions in many pieces of India are generally excellent for stevia development. Major state that produced steavia leaf are Maharashtra, Punjab, Karnataka, Chhattisgarh, Madhya Pradesh, and Andhra Pradesh are major Stevia growing states in India. Gradually stevia farming is picking up in Uttar Pradesh. Stevia plants love to grow in full sun. It is grown best in semi-humid locations with acidic, well-draining soil and environments especially in hot climates. It can be grown in a wide variety of soils but it gives the best outcome when planted in sandy soil to loamy soil with a well-draining system and high organic content. Stevia doesn't grow well in saline soils as it is harmful to its proper development. The pH ranging from 6.0 to 7.5 is best for the proper growth of the stevia plant. The group of Stevia has around 154 species; six species are by and large utilized that are Stevia eupatoria, Stevia ovata, Stevia plummerae, Stevia salicifollia, Stevia serrata, and Stevia rebaundiana. From every one of these, Stevia rebaundiana is the one with important improving properties. The property of the species that brought up the plant was the uncommon sweet trial of the leaves and watery concentrates. Stevia is known for its therapeutic properties. Removes from stevia leaves have been utilized for a seriously lengthy timespan as a restorative spice in the traditional treatment of diabetes. When diverged from sugar, stevia utilization before dinners brings about far lower after-feast glucose and insulin levels. Stevia leaves contain more than eight different steviol glycosides that are Stevioside, Rebaudioside A, C, D, E, and F, Steviolbioside, Dulcoside A. It has cell reinforcement, antimicrobial, calming, hostile to growth, antifungal, against diarrheal, immunomodulatory, antimicrobial, against hypertensive, and hypoglycemic properties. The following are the medical advantages of Stevia; The expanding mindfulness about the clinical benefits of stevia over sugar has provoked an overall augmentation in its interest because of which it is right now become economically across the world. The vast majority of the low-calorie sugars accessible in the market are fake, so the premium of regular sugars like stevia is growing worldwide. Numerous different components influencing the overall interest of stevia are the evolving ways of life, care to be solid, superfluous pay rates, government drives on reducing the sugar utilization, and the need to fight clinical issues like corpulence and diabetes.Stevia is utilized as a solid choice to added sugar in different food things and beverages. Despite the fact that stevia is 200 to multiple times better than standard sugar, so less amount can be used to improve rewards and food.By picking the normal pleasantness and loveliness of stevia, clients can assist people with keeping up an invigorating and nature-accommodating eating routine and that is something that purchasers, wellbeing specialists, and food producers would like.

II. UNIT OPERATION FOR ISOLATION OF STEVIOL GLYCODISE

1. ADSORPTION

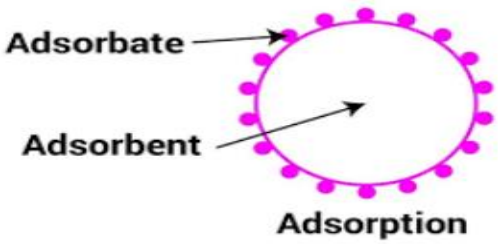
Adsorption is the process through which a substance, originally present in one phase, is removed from that phase by accumulation at the interface between that phase and a separate (solid) phase. Adsorption is one of the most important surface processes.Adsorption is a surface phenomenon. It occurs due to the imbalance of forces at the surface of a material.This lead to formation of bonds (Covalent, ionic, Vander Waals, Hydrogen bonds) between the surface molecules (adsorbents) and the molecules in the fluid phase (adsorbate)

Physisorption

Adsorption in which the forces involved are intermolecular (i.e., van der Waals, hydrogen bonding) of the same kind as those responsible for the non- ideality of real gases and the condensation of vapours etc. , and which do not involve a significant change in the electronic orbital patterns of the species involved is called physisorption.

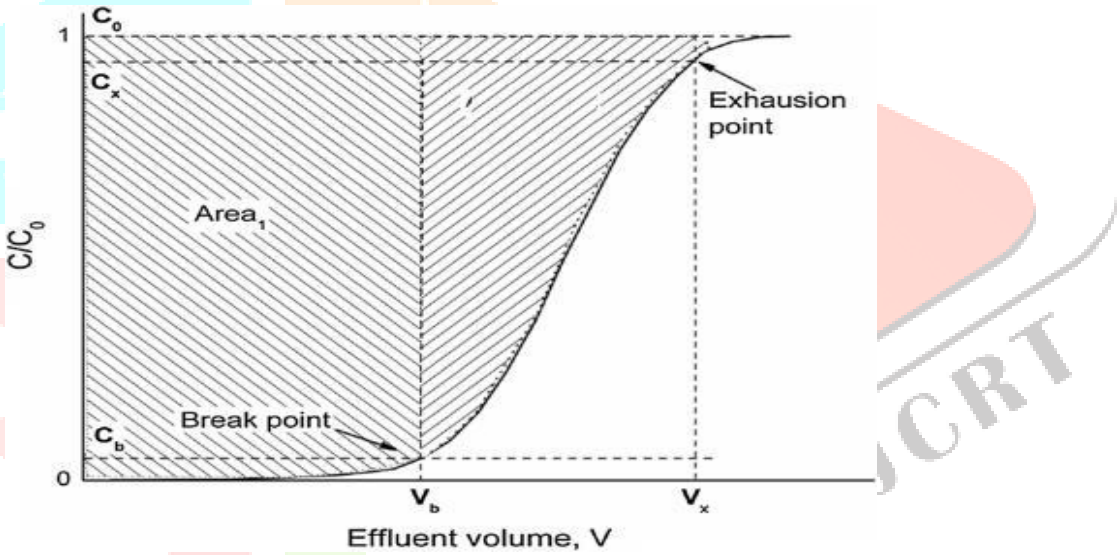
Chemisorption

A chemical process in which a reacting molecule forms a definite chemical bond with an unsaturated atom, or a group of atoms (an active centre) on a catalyst surface, and electron transfer is involved is known as chemisorption.



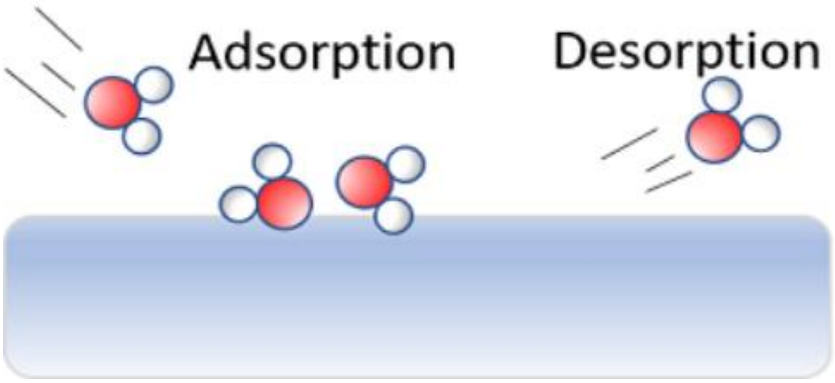
Effect Of Bed Height

The larger the initial concentration, the faster the breakthrough.



2. DESORPTION

Desorption is reverse process of adsorption and this active compound get mix with elute and separate out. It is a process whereby a substance is released from or through a surface. The process is the opposite of sorption (that is, either adsorption or absorption) . This occurs in a system being in the state of sorption equilibrium between bulk phase (fluid, i. e. gas or liquid solution) and an adsorbing surface (solid or boundary separating two fluids).In chemical separation processes, stripping is also referred to as desorption as one component of a liquid stream moves by mass transfer into a vapor phase through the liquid- vapor interface



For elution different concentration of Ethanol solution used.

In chemical separation processes, stripping is also referred to as desorption as one component of a liquid stream moves by mass transfer into a vapor phase through the liquid-vapor interface.

IV. Sample Testing By UPLC

UPLC is a modern technique which gives new direction for liquid chromatography UPLC refers to ultra performance liquid chromatography, which enhance mainly in three areas: "speed, resolution and sensitivity. Ultra performance liquid chromatography (UPLC) applicable for particle less than 2 µm in diameter to acquire better resolution, speed, and sensitivity compared with high- performance liquid chromatography (HPLC).

4.1 Principle and working of UPLC

The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase. The specific intermolecular interactions between the molecules of a sample and the packing material define their time " on- column". Hence, different constituents of a sample are eluted at different times. Thereby, the separation of the sample ingredients is achieved. A detection unit (e. g. UV detector) recognizes the analytes after leaving the column. The signals are converted and recorded by a data management system (computer software) and then shown in a chromatogram. After passing the detector unit, the mobile phase can be subjected to additional detector units, a fraction collection unit or to the waste. In general, a HPLC system contains the following modules: a solvent reservoir, a pump, an injection valve, a column, a detector unit and a data processing unit . The solvent (eluent) is delivered by the pump at high pressure and constant speed through the system. To keep the drift and noise of the detector signal as low as possible, a constant and pulse less flow from the pump is crucial. The analyte (sample) is provided to the eluent by the injection valve.

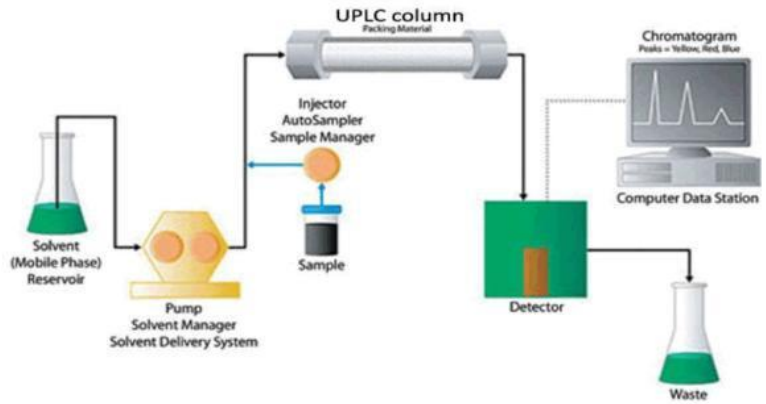


FIG. 1. **Working of UPLC.**

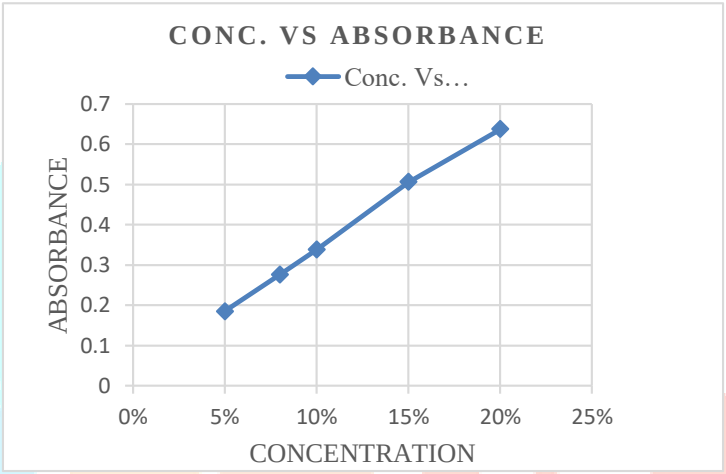
4.2 Column used in UPLC

The column represents the heart of any HPLC system. It is responsible for the adequate separation of the sample ingredients. The separation efficiency correlates with the column inner diameter, the length of the column and the type and particle size of the column packing material. Depending on the desired application, numerous HPLC columns are commercially available. Different packing materials support different separation mechanisms–common are materials for normal-phase, reversed-phase, size exclusion, ion exchange, affinity, chiral, or hydrophilic interaction HPLC.

V. Result and Discussion

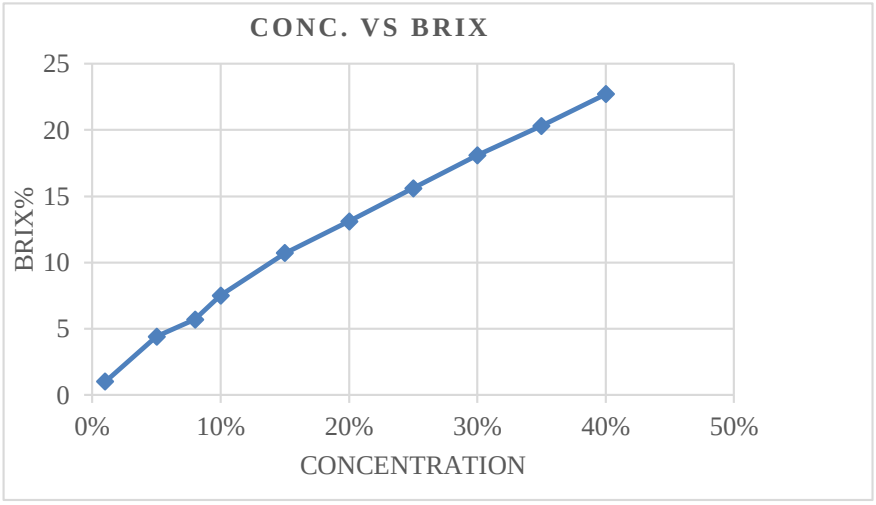
1. Spectrophotometer Analysis of Stevia leaf done on different conc.(5%, 8%, 10%, 15%, 20%.)

S. No.	Con.	max wavelength	Absorbance
1	5%	425	0.185
2	8%	425	0.276
3	10%	425	0.338
4	15%	425	0.506
5	20%	425	0.637



3. Spectrophotometer Data of Different Con. (1%, 5%, 8%, 10%, 15%, 20%, 25%,30%,35%,40%) in 3 ML measurement of . Brix.

s.no.	Concentration	Water	MF (Micrometer)	Brix %
1	1%	3 mL	30	1
2	5%	3 mL	150	4.4
3	8%	3 mL	240	5.7
4	10%	3 mL	300	7.5
5	15%	3 mL	450	10.7
6	20%	3 mL	600	13.1
7	25%	3 mL	750	15.6
8	30%	3 mL	900	18.1
9	35%	3 mL	1050	20.3
10	40%	3 mL	1200	22.7

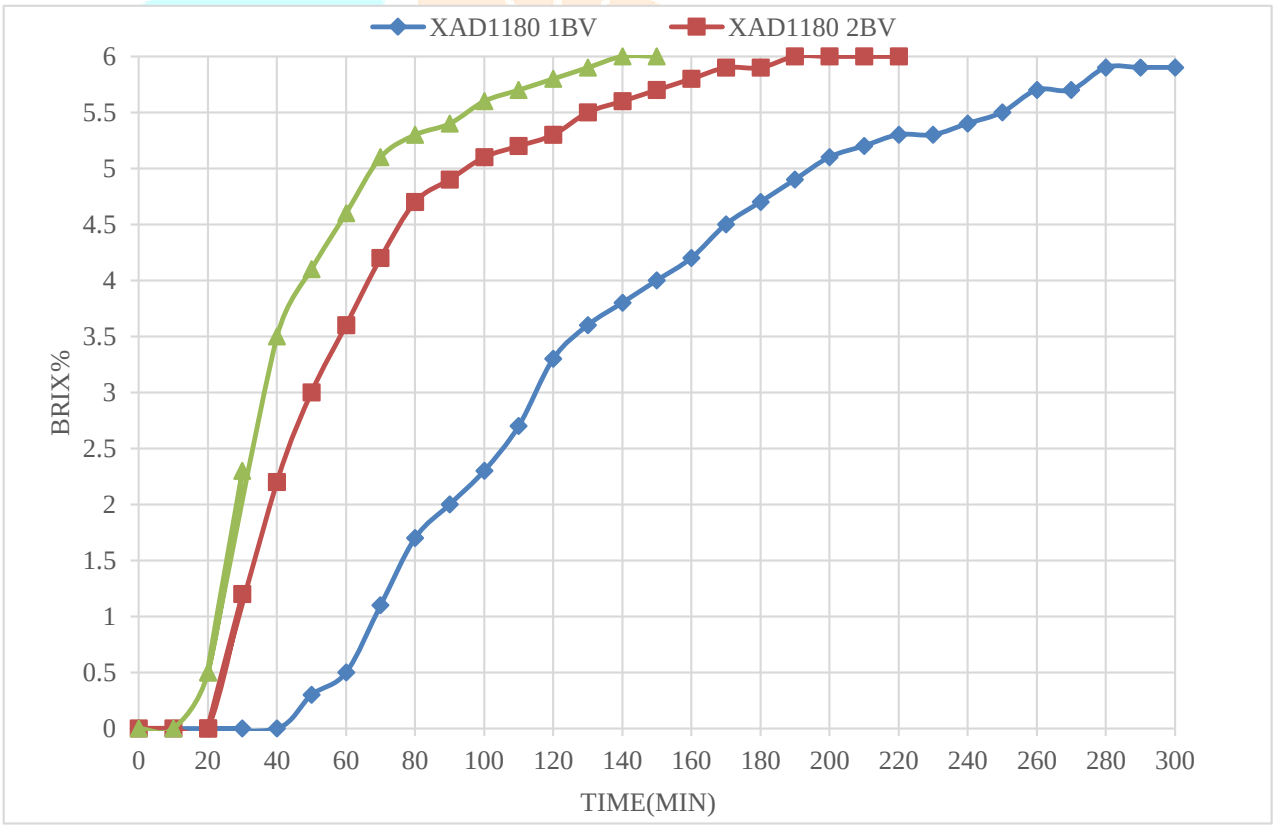


4. Column filed with for isolation of Stevoil Glycoside from stevia leaf Concentrate. SF Concentrate diluted in 1:10 ratio with water and 15 ml resin Bed prepared.

Feed Properties:

Brix- 7.8
Salinity-70.4 ppm
pH- Neutral
TDS-99.8ppm
Conductivity- 141.8 microS/cm

XAD1180 Time Vs. Brix



Resin	BV	Break Point(Min)	Exhaustion Point(min)
XAD 1180	1	45	295
	2	25	210
	3	18	150

Bed Height- 13.7cm Column Height-65.5cm

Eluted samples (10%, 20%, 30%, 40%, 50%, 60% and 100%) were mixed of two batch. eluted sample of 10%, 20%, 30% and 40% runs with Rotary evaporator. Samples were dried lyophilizer and sent for testing.

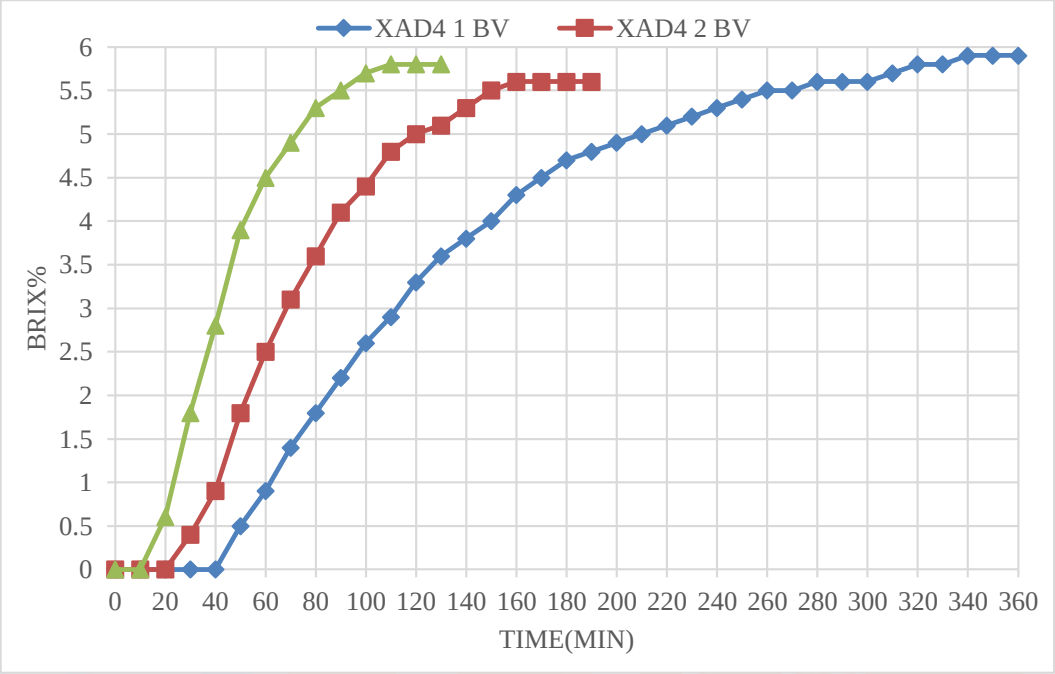
5.Column filed with resin and runs with XAD 4, XAD16 and XAD7 1, 2, 3 BV volume. MF Concentrate diluted in 1:10 ratio and 15 ml resin Bed prepared.

Feed Properties:

Brix- 7.8
Salinity-70.1ppm
pH- Neutral

TDS-98.2 ppm
Conductivity-144.5microS/cm

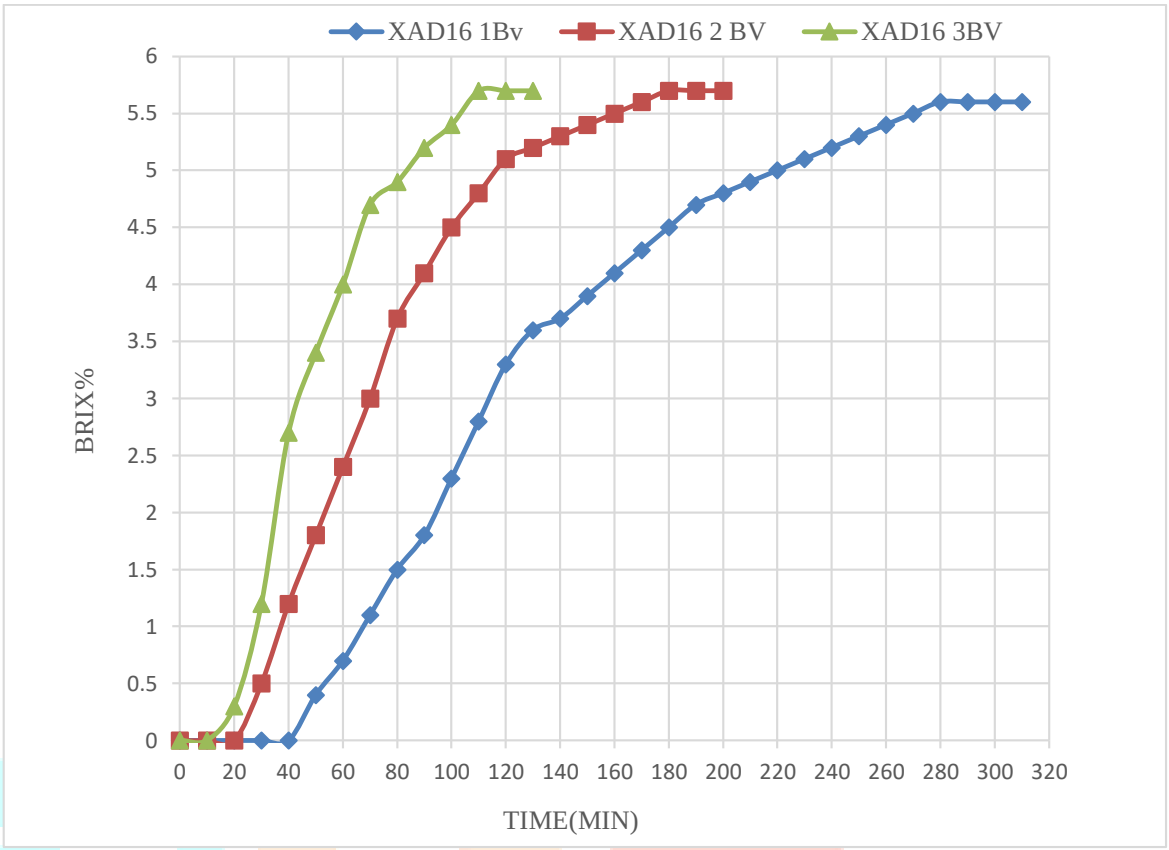
Time XAD 4 Vs. Brix



Resin	BV	Break Point(Min)	Exhaustion Point(min)
XAD 4	1	45	360
	2	25	190
	3	15	130

Bed Height- 16.4cm
Column Height-65.5cm

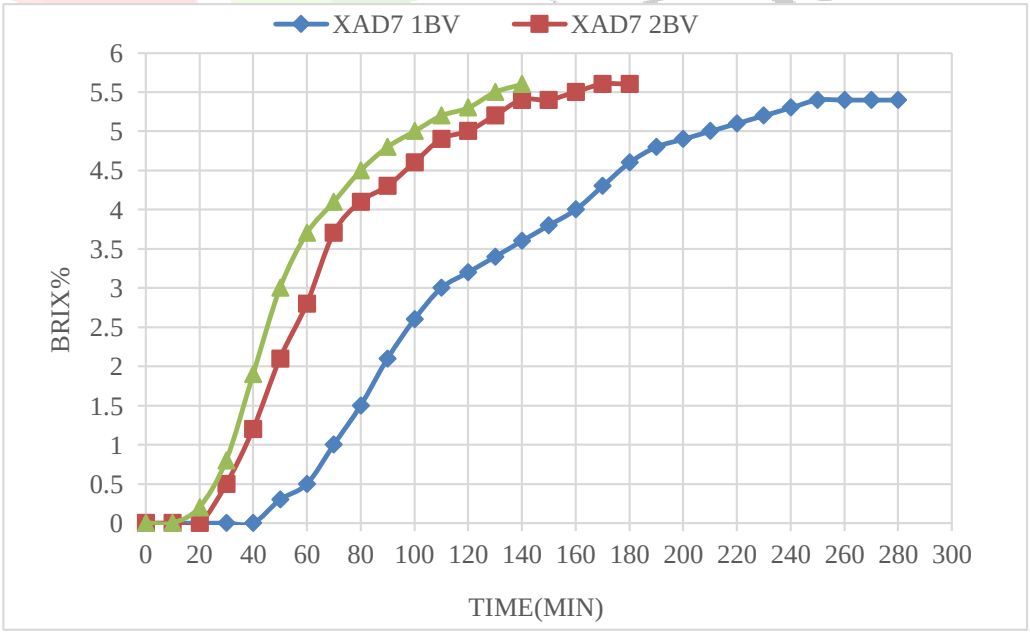
XAD16 Time Vs. Brix



Resin	BV	Break Point(Min)	Exhaustion Point(min)
XAD 16	1	45	300
	2	25	200
	3	18	130

Bed Height-15.9 cm. Column Height-65.5cm.

XAD7 Time Vs. Brix



Resin	BV	Break Point(Min)	Exhaustion Point(min)
XAD 7	1	45	280
	2	30	180
	3	20	150

Bed Height- 16.3cm Column Height-65.5cm

.All the eluted samples (10%,20%,30%,40%,50%,60%and 100%) are collected.
.sample of 10%,20%,30% and 40% runs with Rotary evaporator.
.Samples was dried in lyophilizer and sent for testing.

6.For removal of colour Anion and Cation Column of 40ml prepared and 550 ml stock solution prepared of 1:10 MF concentrate and water for further treatment.

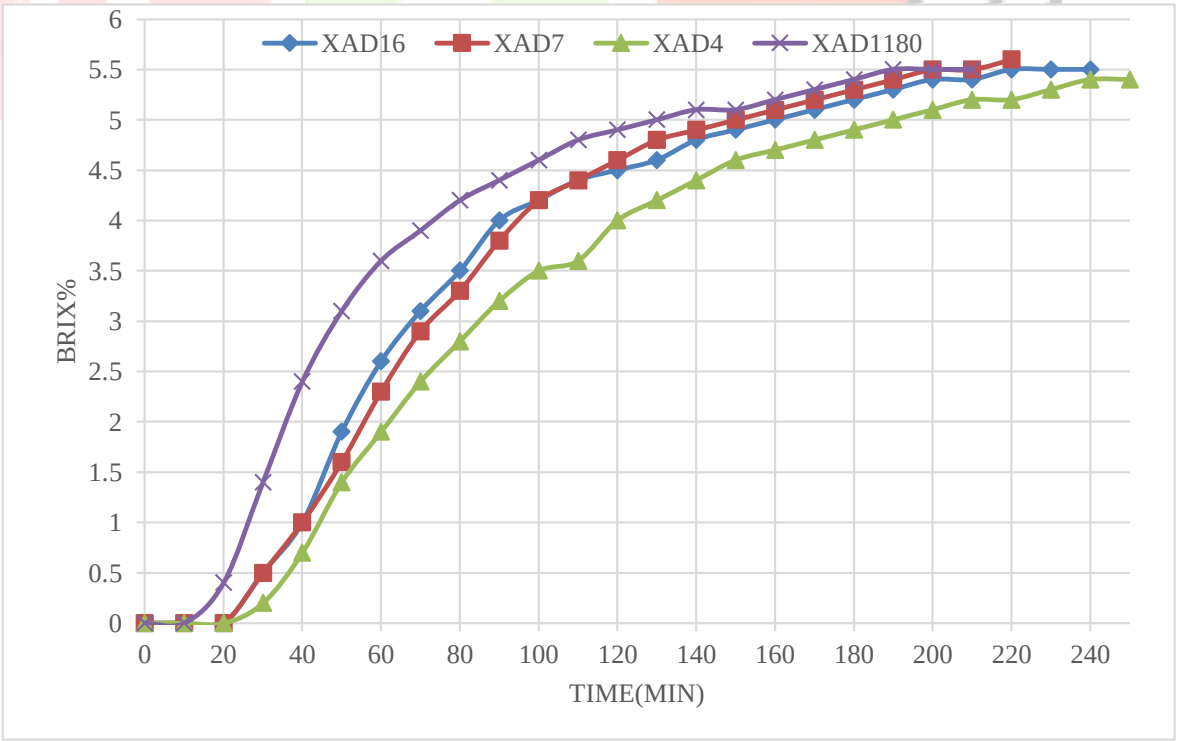
Feed Properties:

Brix- 7.8
TDS-99.7ppm
Salinity-69.6ppm
Conductivity-139.6microS/cm
pH- Neutral

.550 ml sol. Passed form anion and cation resin with 1.5BV.

After Anion	After anion then Cation
Brix- 7.7	Brix- 7.4
TDS-99ppm	TDS- 84.3ppm
Salinity-65.6ppm	Salinity- 60.3ppm
Conductivity-139.9microS/cm	Conductivity -84.3microS/cm
pH- Basic(around 10)	pH- Neutral

.90 ml of sol. Passed form each resin(XAD 4,7,16 and 1180) with 2 Bed Volume,
.Elution done with 40%,60% and 100% and sample collected.
.Desorption done with 15ml of each concentration



Resin	BV	Break Point(Min)	Exhaustion Point(min)
XAD 4	2	30	250
XAD 7	2	25	220
XAD 16	2	25	215
XAD 1180	2	20	210

Break Point and Exhaustion point of all resin

Resin	BV	Break Point(Min)	Exhusion Point(min)
XAD 4	1	45	360
	2	25	190
	3	15	130
XAD 7	1	45	280
	2	30	180
	3	20	150
XAD 16	1	45	300
	2	25	200
	3	18	130
XAD 1180	1	45	295
	2	25	210
	3	18	150

Highest and lowest point:

Resin	BV	Break Point(Min)	Exhaustion Point(min)
XAD 4	1	45	360
XAD 7	1	45	280
XAD 16	1	45	300
XAD 1180	1	45	295

XAD 4	2	25	190
XAD 7	2	30	180
XAD 16	2	25	200
XAD 1180	2	25	210

XAD 4	3	15	130
XAD 7	*3*	*20*	*150*
XAD 16	*3*	*18*	*130*
XAD 1180	*3*	*18*	*150*

Outcome:

For 1 BV XAD 4 has highest exhaustion point. and break point same for all.

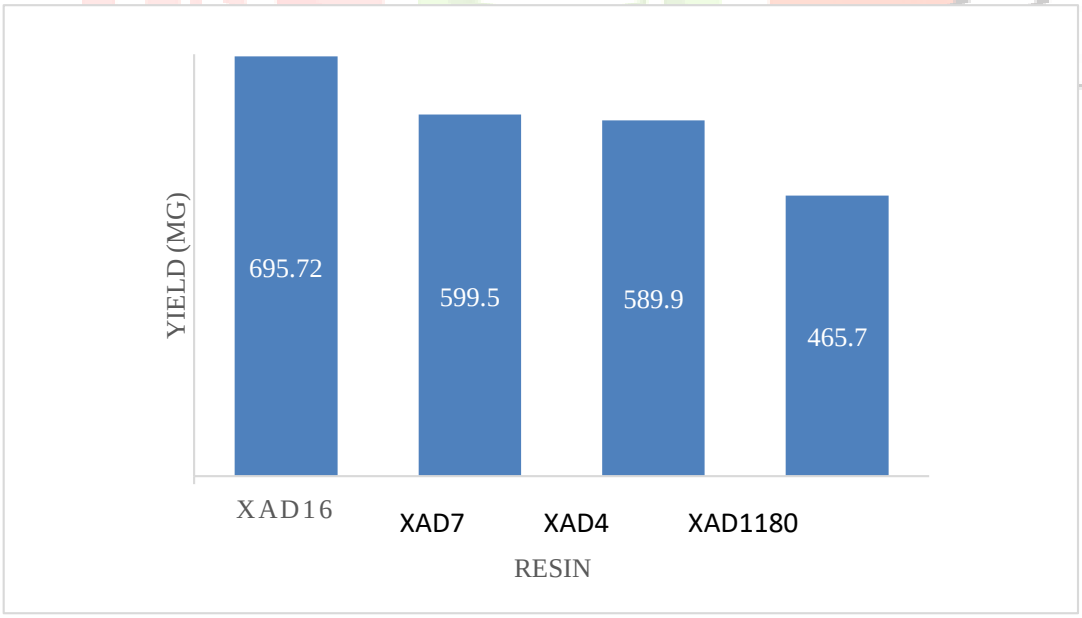
For 2BV XAD16 has highest exhaustion point and For XAD 7 has highest break point.

For 3BV XAD7 has highest exhaustion point and break point

VI . Yield of all resins

After anion and cation

Resin	Flow Rate	Ethonal %	Recovery
	BV		(mg)
XAD16	2	40%	390.9
		60%	243.9
		100%	60.92
			695.72
XAD7	2	40%	434.9
		60%	137.5
		100%	27.1
			599.5
XAD4	2	40%	307.4
		60%	133.4
		100%	24.9
			465.7
XAD1180	2	40%	443.2
		60%	140.6
		100%	6.1
			589.9



Outcome:

A. With 40% Elution has highest yield in the XAD 4,7,16and 1180 resin.
B. In XAD1180 with 40% Highest yield
C. Overall Yield highest in XAD16 with 2BV.

Resin	Flow Rate	Et %	Recovery	Flow Rate	Et %	Recovery	Flow Rate	Et%	Recovery	Total(mg)
	BV		(mg)	BV		(mg)	BV		mg	
XAD4	1	10%	24.8	2	10%	30	3	10%	21.2	
		20%	52.9		20%	35.5		20%	38.8	
		30%	128.2		30%	83.3		30%	82.3	
		40%	126.5		40%	112.4		40%	117.7	
			332.4			261.2			260	853.6 mg
XAD16	1	10%	25.9	2	10%	30	3	10%	20.3	
		20%	20.2		20%	35.9		20%	27.8	
		30%	161.1		30%	83.3		30%	95.2	
		40%	227.1		40%	112.4		40%	142	
			434.3			261.6			285.3	981.2 mg
XAD7	1	10%	10	2	10%	20.7	3	10%	21.9	
		20%	38.6		20%	116.8		20%	81.6	
		30%	38.1		30%	38.9		30%	37.1	
		40%	35.5		40%	35.9		40%	134.9	
			122.2			212.3			275.5	610 mg
	BV		(mg)	BV		(mg)	BV		mg	
XAD1180	1	10%	129.6	2	10%	51.4	3	10%	40.4	
		20%	40.4		20%	167.9		20%	144.8	
		30%	480.2		30%	382		30%	305.8	
		40%	176		40%	270.5		40%	332	
			826.2			871.8			823	2521

*Note- For XAD1180 yield is of two batches

Outcome:

A. With 40% Elution has highest yield in the XAD 4,7and 16 resin beside in XAD 1180 30% Elution has highest yield
B. in XAD 16 for 1BV has highest yield.

VII. ACKNOWLEDGMENT

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VIII. References

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