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Review Article

**ION EXCHANGE CHROMATOGRAPHY**Feba.G<sup>\*1</sup>, Nishad V.M <sup>\* 2</sup>, Prasobh G R<sup>3\*</sup>

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**Abstract:**

*Ion exchange chromatography is a chromatography process that separates ions and polar molecules based on their affinity to the ion exchanger. Ion exchange chromatography is probably the most powerful and classic type of liquid chromatography. It can also used for almost all kinds of charged molecule including large proteins, small nucleotides and amino acids. This topic includes background, theory, application and instrumentation. It is the most effective method for water purification. Inorganic ions also can be separated by ion exchange chromatography. The present review summarizes the instrumentation, validation method and application of ion exchange chromatography.*

*Keywords; Chromatography, Anions, Cations, Ion exchange resins*

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**INTRODUCTION:**

A form of column chromatography to separate, identify, and quantify the compounds. Developed in 1970s. The widely used analytical separation technique.

**CHROMATOGRAPHY**

Chromatography is a technique which separates components in a mixture due to the differing time taken for each component to travel through a stationary phase and carried through it by a mobile phase. It is technique used for separation, purification, identification and extraction of compound. It can consist of two phases stationary phase and mobile phase.

Stationary phase is constant phase or column packaging material. Mobile phase is movable phase. The basic principle of chromatography is based on adsorption partition chromatography.

**Adsorption chromatography** – The affinity to molecules towards stationary phase is known as adsorption chromatography.

**Partition chromatography**- The molecule can move in two phases of liquid is known as partition chromatography. It is important for qualitative and quantitative analysis.

**TYPES OF CHROMATOGRAPHY**

- Based on modes of chromatography
  1. Normal phase mode
  2. Reverse phase mode
- Based on principle of separation
  1. Adsorption chromatography
  2. Ion exchange chromatography
  3. Partition chromatography
  4. Size exclusion
- Based on elution technique
  1. Isocratic separation
  2. Gradient separation
- Based on the scale of operation
  1. Analytical HPLC
  2. Preparative HPLC
- Based on the type of analysis
  1. Qualitative analysis
  2. Quantitative analysis

**DIFFERENT TYPES OF CHROMATOGRAPHIC METHODS**

- Paper chromatography
- Liquid chromatography
- Gas chromatography
- High performance liquid chromatography

**ION EXCHANGE CHROMATOGRAPHY INTRODUCTION**

Ion exchange chromatography is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids. Cations or Anions can be separated using this method.

Ion exchange chromatography is a part of ion chromatography which is an important analytical technique for the separation and determination of ionic compounds, together with ion partition or interaction and ion-exclusion chromatography. Ion chromatography separation is based on ionic or electrostatic interactions between ionic and polar analytes, ions present in the eluent and ionic functional groups fixed to the chromatographic support.

Two distinct mechanisms as follows; ion exchange due to competitive ionic binding (attraction) and ion exclusion due to repulsion between similarly charged analyte ions and the ions fixed on the chromatographic support, play a role in the separation in ion chromatography.

Ion exchange chromatography can be defined as a reversible process in which ions of same sign are exchanged between solid and liquid, a highly insoluble body in contact with it. The soil is known as cation-anion exchange chromatography.

Ion exchange chromatography, which is also known as adsorption chromatography, is a useful and popular method due to its;

- High capacity
- High resolving power
- Mild separation conditions
- Versatility and wide spread applicability
- Tendency to concentrate the sample relatively low cost



## PRINCIPLE

The principle of separation is thus by reversible exchange of ions between the target ions present in the sample solution to the ions present on ion exchangers.

Ion exchange chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte. The ion exchanger consists of an inert support medium coupled covalently to positive (anion exchanger) or negative (cation exchanger) functional groups. To these covalently bound functional groups the oppositely charged ions are bounded (mobile counter ion), which will be exchanged with like charge ions in the sample having charge magnitude more than the ions bounded to the matrix. Thus, if anion exchange chromatography is performed, negatively charged sample components will interact more with the stationary phase and will be exchanged for like charged ions already bounded to the matrix. The ion exchange separations are mainly carried out in columns packed with an ion exchanger.

There are two types of ion exchanger, namely

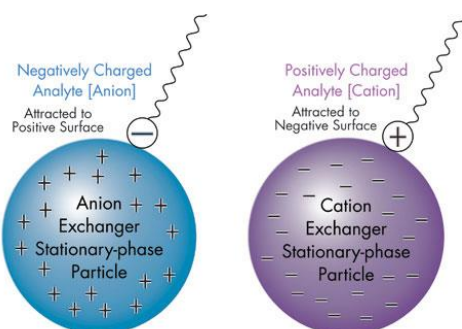
1. Cationic exchangers
2. Anionic exchangers

### Cationic exchangers:

It possesses negatively charged groups and these will attract positively charged groups. These exchangers are also called acidic ion exchange materials since their negative charges result from the proteolysis of acidic groups.

### Anionic exchangers:

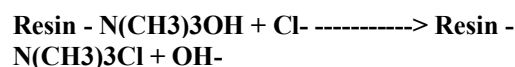
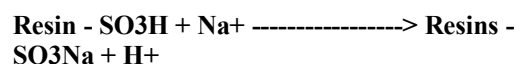
It possesses positively charged groups and these will attract negatively charged molecules. This exchanger is termed as basic ion exchange materials since their positive charges generally result from the association of protons with basic groups. Based upon the affinity of ions towards the matrix the ions like cation and anion are separated. The ions that have less affinity towards matrices will elute first and the ions that have more affinity towards matrices it will elute later.



## ION EXCHANGE MECHANISM

Five distinct steps:

1. Diffusion of the ion to the exchanger surface. This occurs very quickly in homogeneous solutions.
2. Diffusion of the ion through the matrix to the exchanger site. This is dependent upon the degree of cross linkage of the exchanger and the concentration of the solution.
3. Exchange of ions at the exchange site occurs. This occurs instantaneously in an equilibrium process.
4. Diffusion of the exchanged ion through the exchanger to the surface.
5. Selective desorption by the eluent and diffusion of the molecule into the external solution takes places.



## ION EXCHANGERS

Ion exchange processes are used to separate and purify metals. Including, separating uranium from plutonium and other actinides.

There are three classes of ion exchangers;

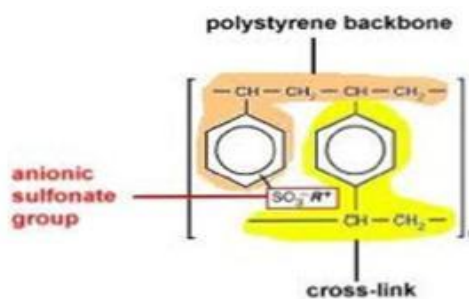
1. Resins
2. Gels
3. Inorganic exchangers

### RESINS

Resins are amorphous particles of organic materials, which are composed of polystyrene and divinyl benzene.

- Polystyrene contains sites for exchangeable functional groups.
- Divinyl benzene acts as cross-linking agents and offers adequate strength. i.e., mechanical stability.

Ion exchange resins are used for separation of small molecules.



Structure of polystyrene

## CLASSIFICATION OF ION EXCHANGE RESINS

## A. According to chemical nature

- Strong cation exchange resin – sulphonic acid
- Weak cation exchange resin – Carboxy methyl compound
- Strong anion exchange resin – Quaternary ammonium compound
- Weak anion exchange resin – Diethyl amino ethyl compound

## B. According to the source

1. Natural
  - Cation – Zeolytes, clay
  - Anion – Dolomite
2. Synthetic
  - Inorganic and organic resins

Organic resins are polymeric resin matrix. The resin composed of Polystyrene and Divinyl benzene.

## C. According to the structure

## a. Pellicular type with ion exchange film

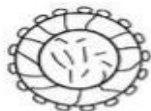
Particle size of 30-40 micron with 1-2-micron film thickness



Pellicular Type

- Very low exchange capacity
- Ion exchange efficiency is 0.01-0.1meq/g of ion exchange resin

## b. Porous resin coated with exchanger beads



Porous type Resin

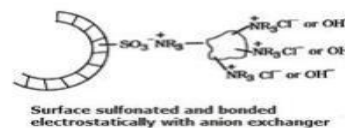
- Particle size of 5-10 micrometer
- Porous and highly efficient
- Ion exchange efficiency is 0.5-2meq/g of ion exchange resin

## c. Macroreticular resin bead



Macro Reticular bead

- Highly efficient
  - Very low Not exchange capacity
- d. Surface sulfonated and bonded electrostatically with anion exchanger



Surface sulfonated and bonded electrostatically with anion exchanger

- Low efficient
- Low exchange capacity

## GELS

- Ion exchange gels are used for the separation of large molecules like proteins, nucleic acids.
- Cellulose and dextran ion exchangers which are polymers of sugar glucose possess large pore sizes and lower charge densities.
- They are much softer than polystyrene resins, dextran and its relatives are called as gels.

## INORGANIC EXCHANGERS

- The combinations of hydrous oxide of highly charged ions, with one oxide more acidic than the other have been found to have ion exchanging properties.
- The amorphous precipitates have higher exchange capacities than the crystalline compounds, because of the greater surface area of the former type of compounds.
- Inorganic exchangers are employed when separations involving harsh chemical conditions such as high temperature, high radiation level, strong basic solution or powerful oxidizing agent.
- E.g., Titanium arsenite has been used to absorb alkaloids.

Hydrous antimony peroxide has been used to study exchange equilibrium of Ka and Rb ions with hydrogen and other ions.

## PROPERTIES OF ION EXCHANGE RESIN

- It must be chemically stable
- They are almost insoluble in water, benzene, ether etc.
- It must contain sufficient no of ion exchange groups.
- It should have a sufficient degree of cross linking.
- The swollen resin must be denser than water.

## PRACTICAL REQUIREMENTS

1. Column material and dimension
2. Type of ion exchange resin and physical characteristics
  - a. Type of ions
  - b. Nature of ions
  - c. Efficiency of resin
  - d. Particle size
  - e. Structural type

3. Packing of the column
4. Mobile phase
5. Development of the chromatogram
6. Analysis of the elite
7. Regeneration of the ion exchange resin

#### 1. Column material and dimensions

Columns used in the laboratories are made up of glass. In industries are made up of either high quality stainless steel or polymers which are resistant to strong acids and alkalis. The column dimensions are also important and a length: diameter ratio of 20:1 to 100:1 for higher efficiency can be used.

#### 2. Type of ion exchange resin

- Type of ions – Cations or Anion
- Nature of ions – Strong or Weak
- Efficiency of the resin – It is measured by ion exchange capacity

Ion exchange capacity:

It is the total ion exchange capacity in terms of the exchangeable functional groups expressed as milli equivalents per gram of the exchange resin.

$$\text{m.eq/g} = 1000/\text{eq. wt.}$$

Particle size of the resin - 50- 100 mesh or 100-200 mesh is used.

Structural type of the resin - porous, pellicular

Amount of cross-linking agent present -Which decides swelling of the resin

#### 3. Packing in of the column

- Wet packing method is used.
- Resin + mobile phase – Packing in the column uniformly

#### 4. Mobile phase

- Organic solvents are less useful and they are not used at all.
- Only different strengths of acids, alkalis and buffers are used as eluting solvents.

E.g.: 0.1 N HCl, 1N NaOH, Phosphate buffer, acetate buffer, borate buffer, phthalate buffer etc...

#### 5. Development of the chromatogram and elution

After introduction of the sample, development of the chromatogram is done by using different mobile phases. As mentioned earlier, organic solvents are less useful and only acids, alkalis and buffers of different pH are used.

There are two elution techniques:

- i. Isocratic elution
- ii. Gradient elution

#### Isocratic elution

Same solvent composition is used. i.e., same strength of acid or alkali or buffer.

#### Gradient elution

In gradient elution technique, initially less acidic or basic character is used followed by increasing the acidity or basicity of the mobile phase.

This elution technique is usually used for complex mixtures. The different fractions of the eluent is collected volumewise or timewise and analyzed.

#### 6. Analysis of the elute

Several methods of analysis can be used which depends upon the nature and quantity of the sample.

1. Spectrophotometric method
2. Polarographic method
3. Conductometric method
4. Amperometric method
5. Flame photometric method
6. Radiochemical method
  - Geiger Muller Counter
  - Ionisation Chamber method

After analyzing, similar fractions are mixed in order to get pure ion or compound of each type.

#### 7. Regeneration of the ion exchange resin

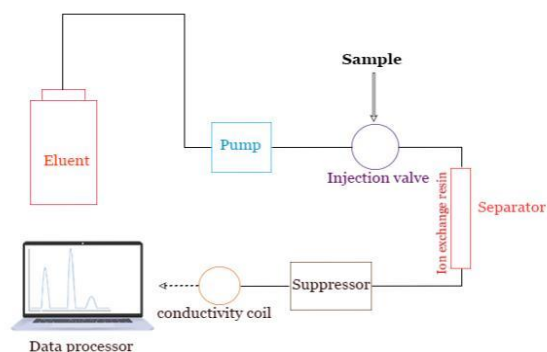
Regeneration makes the used ion exchange resin to be as efficient as a virgin resin.

Regeneration refers to the replacement of the exchangeable cations or anions present in the origin resin. Hence regeneration of the Cation exchange resin is done by the charging the column with strong acid like hydrochloric acid.

Regeneration of anion exchange resin is done by using strong alkali like sodium hydroxide or potassium hydroxide.

## INSTRUMENTATION

Typical IC instrumentation includes: pump, injector, column, suppressor, detector and recorder or data system.



#### Pump

The IC pump is considered to be one of the most important components in the system which has to provide a continuous constant flow of the eluent through the IC injector, column, and detector.

#### Injector



Sample introduction can be accomplished in various ways. The simplest method is to use an injection valve. Liquid samples may be injected directly and solid samples need only to be dissolved in an appropriate solvent. Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 ml of volume with high reproducibility and under high pressure (up to the 4000 psi).

#### Columns

Depending on its ultimate use and area of application, the column material may be stainless steel, titanium, glass or an inert plastic such as PEEK. The column can vary in diameter from about 2mm to 5 cm and in length from 3 cm to 50 cm depending on whether it is to be used for normal analytical purposes, microanalysis, high speed analyses or preparative work.

Guard column is placed anterior to the separating column. This serves as a protective factor that prolongs the life and usefulness of the separation column. They are dependable columns designed to filter or remove particles that clog the separation column.

#### Suppressor

The suppressor reduces the background conductivity of the chemicals used to elute samples from the ion-exchange column which improves the conductivity measurement of the ions being tested. IC suppressors are membrane-based devices which are designed to convert the ionic eluent to water as a means of enhancing the sensitivity.

#### Detectors

Electrical conductivity detector is commonly use.

#### Data system

In routine analysis, where no automation is needed, a pre-programmed computing integrator may be sufficient. For higher control levels, a more intelligent device is necessary, such as a data station or minicomputer.

### WORKING PRINCIPLE OF ION EXCHANGE CHROMATOGRAPHY

This form of chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte.

- ❖ The ion exchangers basically contain charged groups covalently linked to the surface of an insoluble matrix.
- ❖ The charged groups of the matrix can be positively or negatively charged.

- ❖ When suspended in an aqueous solution, the charged groups of the matrix will be surrounded by ions of the opposite charge.
- ❖ In this “ion cloud”, ions can be reversibly exchanged without changing the nature and the properties of the matrix.

### PROCEDURE

Ion exchange separations are carried out mainly in columns packed with an ion exchanger.

- These ionic exchangers are commercially available. They are made up of styrene and divinyl benzene.

E.g.: DEAE-cellulose is an anionic exchanger, CM-cellulose is a cationic exchanger.

- The choice of the exchanger depends upon the charge of particle to be separated. To separate anions “Anionic exchanger” is used, to separate cations “Cationic exchanger” is used.
- First the column is filled with ion exchanger then the sample is applied followed by the buffer. The tris-buffer, pyridine buffer, acetate buffer, citrate and phosphate buffers are widely used.
- The particles which have high affinity for ion exchanger will come down the column along with buffers.
- In next step using corresponding buffer separates the tightly bound particles.
- Then these particles are analyzed spectroscopically.

### ION EXCHANGE TECHNIQUES

There are two methods,

1. Batch method
2. Column method

#### Batch method

- It involves single step equilibrium processes
- Resin + solutions are mixed in a vessel
- Filter the solution
- Only a single portion of the exchange capacity of the resin is utilized
- It is used for softening of water and production of deionized water

#### Column method

- It involves in separation of components of a mixture by selecting different coefficient of resin.
- The difference in selectivity coefficient leads to different migration rates on ion exchange column.
- Chromatography process is classified according to physically state of mobile and stationary phases.

- It involves frontal analysis, elution analysis, displacement development.

### APPLICATIONS

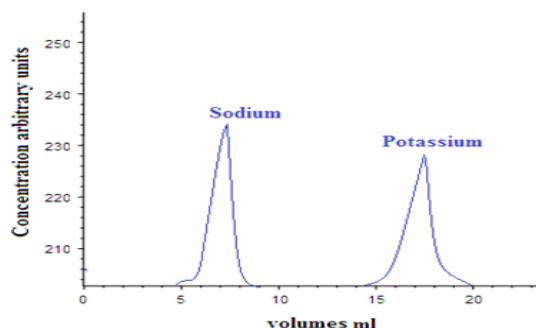
Ion exchange chromatography can be applied for the separation and purification of many charged or ionizable molecules such as proteins, peptides, enzymes, nucleotides, DNA, antibiotics, vitamins and etc. from natural sources or synthetic origin.

Some of its applications are as follows;

#### 1. Separation of similar ions

The ion exchange chromatography is used for separation of similar ions as different ions undergo exchange reactions to different extent.

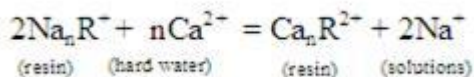
E.g.: A mixture of  $\text{OH}^+$ ,  $\text{Na}^+$  and  $\text{K}^+$  can be separated by using cation exchange resin. Similarly,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$  can be separated by passing through basic anion exchanger.



Graph-1: Separation of potassium from sodium on Dowex 50 x 12 by elution with 0.6 M HCl

#### 2. Softening of hard water

Hardness of water is due to the presence of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and other divalent ions may be removed by passing the hard water through the cation exchanger



charged with  $\text{Na}^+$  ions. Then the following exchange reaction takes place:



The  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  ions from water are retained on the column while  $\text{Na}^+$  ions pass into the solution. These  $\text{Na}^+$  ions are harmless for washing purpose. After using the ion exchange for a time, it becomes inactive. Percolating through it a concentrated solution of  $\text{NaCl}$

when the following reverse reaction takes place can revive its activity.

#### 3. Complete demineralization of water

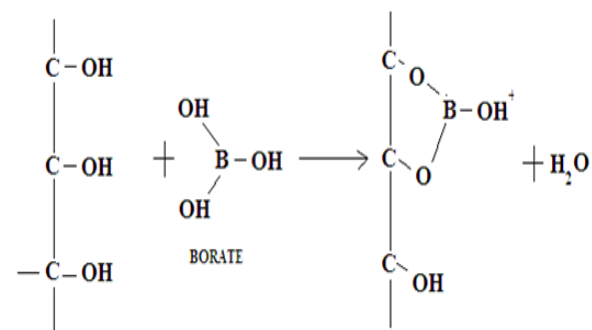
This requires complete removal of ions i.e., both cations and anions. For this, water is passed through an acidic cation exchanger then metallic cations are exchanged with  $\text{H}^+$  ions. The water obtained is then passed through a basic anion exchanger then the anions present in the water are exchanged by  $\text{OH}^-$  of the exchanger. The  $\text{H}^+$  and  $\text{OH}^-$  ions which pass into solution combine to form unionized water. E.g.: Cation exchanger- Sulphonic acid resin is commonly used. Anion exchanger- Strong basic resin is used.

#### 4. Purification of organic compounds

Many natural products extracted in water have been found to contain ions originally present in water. Those ions can be removed by using ion exchange process.

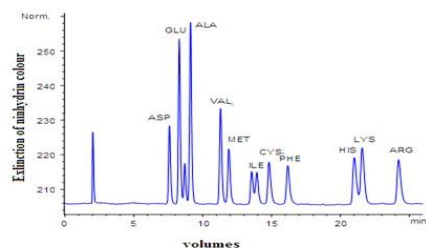
#### 5. Separation of sugars

This method is developed by Khym and Zill in 1951. Sugars are first converted into borate complex and the separation of borate complexes and the separation of borate complexes have been achieved quantitatively on columns by using ion exchange chromatography. In this, disaccharide can be separated from monosaccharides and the individual compounds of hexose, pentose from the mixture can be resolved.



#### 6. Separation of amino acids

Ion exchange methods can be used to separate the complex mixture of 18 amino acids obtained by the acid hydrolysis of proteins. The mixture of amino acids is first introduced on a very short column at pH 2 and eluted with 0.35N sodium citrate buffers at pH 5.25. Acidic and neutral amino acids at first leave the column unseparated and after that other amino acids are separated. Similarly, a mixture of vitamins like vitamin B1, B2, B6, Niacin, Folic acid, B12 etc. can be separated using ion exchange technique.



Graph-2: Separation of amino acids

### 7.Purification and recovery of pharmaceuticals

The process is used for purification and recovery of antibiotics, vitamins, alkaloids, hormones and other chemicals of pharmaceutical importance during their manufacturing process.

### 8.Medicinal importance

Anionic resins are introduced in the treatment of ulcer while cation exchangers have been used to remove  $\text{Na}^+$  from body during the treatment of hypertension and edema. The resins are also used as a diagnostic aid in gastric acidity tests. The resins have been successfully used with other medicinal agents to achieve delayed action dosages.

### 9.Biochemical separations

Used for biochemical separations like some drugs or metabolites from blood, urine or other biological fluids.

### 10.Ion exchange column in HPLC

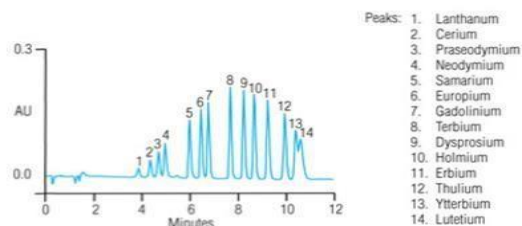
For separation of compounds of mixed nature like acidic and basic substances, ion exchange is used in HPLC

### 11.Concentration of ionic solutions

A cation or anion from a bulk of solution can be adsorbed onto ion exchange resins, after adsorption, it can be eluted by using small volume of eluent.

### 12.Separation of lanthanides

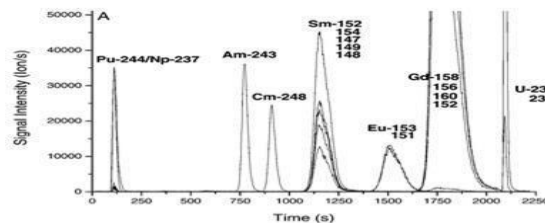
Solution having mixture of lanthanides is passed through a column packed with particles of a suitable ion exchange resin. Cations present in solution undergo exchange with hydrogen with hydrogen ions.



Separation of lanthanides

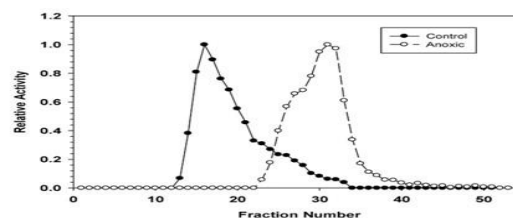
### 13.Separation of actinides

The IEC technique has played a unique role in the discovery on the trans plutonium elements in the actinide series. The power of the method can be judged from the order of elution of lanthanides and actinide ions in the +3 oxidation state from a cation exchange resin column with an aqueous solution of ammonium hydroxyl isobutyrate. In the actinide series also the elution occur in the reverse order of the atomic number due to actinide contraction and this proved that IEC is only way for identifying these elements.



### 14.Removal of interfering radicals

The estimation of  $\text{Ca}^+$  or  $\text{Br}^+$  ions is carried out by the oxalate or sulphate method in which phosphate ion is found to interfere. Therefore, its removal becomes necessary which is achieved by passing a solution of  $\text{Ca}^+$  or  $\text{Ba}^+$  ions through suitable ion exchanger in the column. The process has to be repeated so that the phosphate ions are completely removed. Now, the calcium and  $\text{Ba}^+$  ions held by resin will be removed by using suitable eluent. Finally, these ions are estimated by the usual methods.



Graph-3: Removal of interfering radicals

### 15.Other applications

- For the measurement of various active ingredients in medicinal formulations.
- For the measurement of drugs and their metabolites in serum and urine, for residue analysis in food raw materials.
- For the measurement of additives such as vitamins and preservatives in food and beverages.

### LIMITATIONS

- Column efficiency is less
- It is difficult to achieve control over selectivity and resolution.
- Stability and reproducibility of the columns become questionable after repeated use.



- Nature of exchanging ions is not known.
- The organic matter Fe<sup>3+</sup> occurring in some water which can foul the resin.

#### METHOD OF VALIDATION

During method optimization, all chromatographic parameters were found to prove specificity, precision, linearity, accuracy, robustness, solution and mobile phase stability of fumarate, oxalate, succinate, and tartrate anions.

##### Specificity

Specificity is the ability to unequivocally assess the analyte in the presence of its potential impurities, which may be expected to be present like impurities, degradants, matrix, etc. The specificity of the developed ion-exchange chromatographic method was established in the presence of 11 anions and four active pharmaceutical ingredients (API), namely trifluoroacetic acid (TFA), chloride, nitrate, bromide, phosphate, sulphite, succinate, tartrate, sulphate, oxalate, fumarate and the APIs quetiapine, escitalopram, sumatriptan, and tolterodine.

Drugs were not subjected to forced degradation, as the impurities generated were organic moieties, which do not have any response in the ion-exchange chromatographic method.

##### Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple samplings of the homogenous sample under the prescribed conditions. The precision for quetiapine fumarate, escitalopram oxalate, sumatriptan succinate, and tolterodine tartrate were checked at the 134 µg/mL, 100 µg/mL, 66 µg/mL, and 66 µg/mL anions, respectively, corresponding to the theoretical content anions, i.e. 15.3%, 21.2%, 18.5%, and 31.3% of fumaric acid, oxalic acid, succinic acid, and tartaric acid in quetiapine fumarate, escitalopram oxalate, sumatriptan succinate, and tolterodine tartrate, respectively. Method precision was performed on six different preparations of the test samples. The percentage relative standard deviation of the content of all four anions in the six preparations was calculated.

The intermediate precision of the method was also evaluated by a different analyst, different instrument, and on a different day.

##### Linearity

The linearity of an analytical test procedure is its ability to obtain test results within the given range

which is directly proportional to the concentration of the analyte in the sample. The linearity of the method was checked at seven concentration levels: from 25 µg/mL to 200 µg/mL of fumaric acid, oxalic acid, succinic acid, and tartaric acid. The calibration curve was drawn by plotting the peak areas of all four acids against the corresponding concentrations. The correlation coefficients of the regression lines of the calibration curves were also calculated.

##### Range

The range is estimated via linearity studies.

It can be defined as the determination of highest and lowest limit of the analyte present in the sample that has been indicated to be measurable using the method along with the applicable degree of accuracy, linearity and precision within the stated range of the analytical procedure.

Results collected after the accuracy, precision and linearity analysis were utilised to estimate or establish the range of the developed method or analytical procedure.

##### Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value, and the expected value found. Standard addition and recovery experiments were conducted to determine the accuracy of the quantitation of fumaric acid, oxalic acid, succinic acid, and tartaric acid in quetiapine fumarate, escitalopram oxalate, sumatriptan succinate, and tolterodine tartrate samples. The study was carried out by weighing drug substances to attain 50%, 100%, and 150%. Theoretical concentrations of the anions in their respective prepared drug substances were injected in triplicate at each level. The % recoveries of all four acids were calculated from the slope and y- intercept of the calibration curve obtained.

##### Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage, and the flow rate of the mobile phase was 1.0 mL/min in the method. To study the effect of flow rate on system precision, it was changed by 0.1 units to 0.9 mL/min and 1.1 mL/min, while mobile phase components were held constant and the effect of flow rate was studied. The acetonitrile in the mobile phase composition was 10% in the method. To study the effect of % acetonitrile on the system precision, it was changed by 2% to 8% and 12%, while other

components were held constant and the effect of the change in % acetonitrile was studied. The concentration of sodium carbonate and sodium bicarbonate was 7.5 mM and 2.0 mM in the method. To study the effect of concentration of sodium carbonate and sodium bicarbonate, these were changed by 0.75 units to 6.75 mM and 8.25 mM for sodium carbonate, and by 0.2 units to 1.8 mM and 2.2 mM for sodium bicarbonate, while the other components were held constant and the effect of these changes were studied.

Robustness was not studied for column temperature as the method employs the column equilibration at room temperature in an analytical laboratory.

Solution stability and Mobile phase stability

The solution stability was carried out by keeping both test solutions and reference solutions in tightly capped volumetric flasks at room temperature for 72 h. The sample solutions were analysed at initial, 24 h, 48 h, and 72 h. The stability of the mobile phase was also carried out for 72 h by analysing the freshly prepared reference solutions at initial, 24 h, 48 h, and 72 h. The mobile phase was kept constant during the study.

It can be defined as the determination of highest and lowest limit of the analyte present in the sample that has been indicated to be measurable using the method along with the applicable degree of accuracy, linearity and precision within the stated range of the analytical procedure.

Results collected after the accuracy, precision and linearity analysis were utilised to estimate or establish the range of the developed method or analytical procedure.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value, and the expected value found. Standard addition and recovery experiments were conducted to determine the accuracy of the quantitation of fumaric acid, oxalic acid, succinic acid, and tartaric acid in quetiapine fumarate, escitalopram oxalate, sumatriptan succinate, and tolterodine tartrate samples. The study was carried out by weighing drug substances to attain 50%, 100%, and 150%. Theoretical concentrations of the anions in their respective prepared drug substances were injected in triplicate at each level. The % recoveries of all four acids were calculated from the slope and y- intercept of the calibration curve obtained.

Robustness

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### CONCLUSION:

Ion exchange chromatography is a technique often used in protein purification, water analysis and quality control. It can be used for large proteins, small nucleotides and amino acids. The principle of ion exchange chromatography was that, charged molecules bind electrostatically to oppositely charged groups that have been bound covalently on the matrix.

This method is widely applicable to the analysis of a large number of molecules with high capacity. The technique is easily transferred to the manufacturing scales with low cost. High levels of purification of the desired molecule can be achieved by ion exchange step.

The ion exchange chromatography has been used in the separation of ionic molecules for more than half a century is still used as an useful and popular method for isolation of natural products in modern drug discovery and it continue to expand with development of new technologies.

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