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STUDY ON THE APPLICATION OF ION CHROMATOGRAPHY FOR ANALYSIS AND PURIFICATION

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Abstract: **There are several methods for analysis eg: G.C, HPLC, wet chemical method, enzyme method but ion chromatography becoming the method of choice. It is a well-stable technique for the determination of ions in solution. IC is used in many fields for the determination of ions in solution. because of its high sensitivity of the technique, coupled with the wide dynamic operation range made possible with the modern high capacity stationary phase makes it deal for the analysis of ion in pharmaceutical and drug with the help of ic. The global beverage industry is growing each year with the introduction of new products such as vitamins, fortified water, energy drink, herbal nutrient supplement because of which come many more analytical challenges. These challenges are compounded by continuing new needs to analyze classics and favorites such as sodas, fruits, milk, drinks, alcoholic beverages, and bottled water. This review article provides an overview of the relevant application and focusing on works published in the past year. this topic has been well covered in several books and reviews.**

key word: **ion chromatography, water, pharmaceutical, clinical, beverage**

I. INTRODUCTION:

Chromatography was originally introduced by two English researchers, English agricultural chemist sir HS Thomason. He found that on passage of ammonia sulfate solution through soil som or all the ammonia was replaced by the calcium ion. He called this exchange chromatography. In 1935 it was advance Adam and holmes published the first paper on the synthesis of ion exchange resin. an Important In modern form, ion-exchange chromatography was introduced by Hamish small et al at Dow chemicals in 1975. ggerdeat published a method for anion chromatography in 1979 and this was followed by a similar method for cation chromatography in 1980. the full information on the development of IC over 40 years through the chemical and instrumental point is there on an advance in the ion chromatography (Thermo Science, 20013).

IC is an important analytical technique for the separation and determination of ionic compounds. it is based on ionic interaction 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 and ion exclusion due to repulsion between similarly charged analyte ions and ions fixed on the chromatographic support plays a role in the separation of ion chromatography. In the early 20th century, zeolite columns were used to remove interfering calcium and magnesium ions from the solution to permit the determination of sulfate. The ionic separation procedure was used in the Manhatten project to purify the concentrated radioactive needed to make an atom bomb. Peterson and sober reported in 1956 a chromatographic method based on ion exchange to separate protein.

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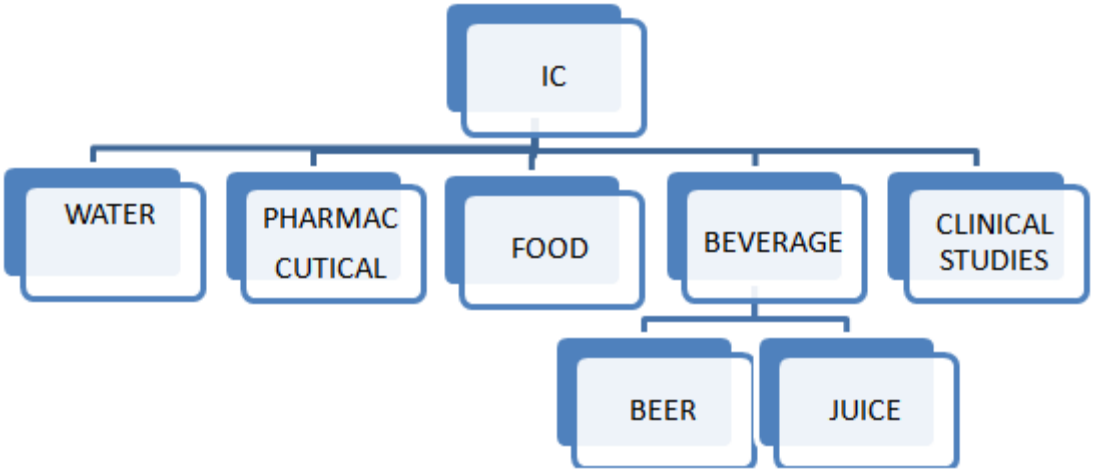

1.1) Application in water analysis

EPA Regulatory Status for Ions Found in Drinking Water Commonly Analyzed by Ion Chromatography

Ions found in drinking water	Regulated contaminant	Maximum contaminnt limit mg/l	Secondary standard	Recommended Concentration Limit (mg/L)	Unregulated Component
floride	x	4	x	2	
chlorite	x	1			
Cynide(free)	x	0.2			
bromate	X	0.01			
Chloride			x	250	
Nitrite(asN)	x	1			x
Bromide					
Nitrate(asN)	x	10			
Chlorate					X
Haloacetic acid	x	0.06			
Arsenic	x	0.01			
phosphate					X
Chromium (total)	x	0.01			
selenium	x	0.05			
Iodide					
sulfate					x
lithium			x	250	
Sodium					x
Ammonium					X
Potasium					X
Rubidium cesium					X
Magnesium					X
Calcium					X
Strontium					X
Barium					X

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The water that we drink may contain nitrate, which changes into nitrite in the body can lead to many health issues ie. methemoglobinemia, lower blood pressure. nitrates dissolve in water from the air as well as via direct contamination of groundwater as well as from rainwater sources in areas heavily contaminated with atmospheric nitrate. In most of the developed world, the major source of nitrate and nitrite in the water supply is agricultural runoff as well as direct groundwater contamination in agricultural areas. Nitrate-based fertilizer is commonly the source of nitrogen in industrialized agriculture. nitrates are highly mobile in groundwater. in agricultural areas, groundwater is replaced by bottled water for living. Spectrophotometric and ion-selective electrode analytical methods are widely used for the determination of both nitrate and nitrite in drinking water. nowadays IC has become a generally preferred alternative to these methods for water analysis laboratories equipped with a full range of analytical instrumentation, given the fact that IC allows for the determination of not just nitrite and nitrite, but a whole range of ionic contaminants commonly found in drinking water.

there are many ways to design a stationary phase one of which is to use more polar polymer backbones for the preparation of anion exchange materials. An example of this is the IC SI-50 4E column from Shodex (also available as an OEM material sold by Metrohm under the name Metrosep A Supp 5). This anion exchange material makes use of a hydrophilic crosslinked polyvinyl alcohol polymer backbone with ion-exchange sites introduced via reaction with epichlorohydrin and subsequent reaction with a tertiary amine. Columns based on this polymer architecture exhibit excellent nitrate peak shape. The only disadvantage of this polymer architecture is that the polymer matrix is significantly softer than polymers based on methacrylate or styrene monomers. As a consequence, the recommended flow rate for a 4-mm ID column (0.7 mL/min) is very close to the maximum recommended flow rate (0.8 mL/min). As a result, columns based on this chemistry platform are not as robust as more conventional IC phases. For example, the column bed is relatively easily disturbed by fluctuations in flow rate associated with normal operating conditions, such as when the pump loses prime or during initial instrument startup.

Designing the stationary phase is not the challenge in quantitative analysis of nitrate in the water sample. the difficulty is in column selection about nitrite quantitation stems from two factors: the relatively low concentration of nitrite in drinking water samples and the general tendency of nitrite to elute just after chloride. The combination of these two features can make it difficult to quantitate trace levels of nitrite, given the fact that chloride is often 1000 e10,000 times more concentrated than nitrite in drinking water samples. As a consequence, the optimum column choice for quantitation of nitrite is a column that maximizes the separation from chloride while at the same time providing good peak shape for chloride under moderately overloaded conditions. Generally, columns such as the IonPac AS22, shown in, or columns based on polyvinyl alcohol substrates provide the the best combination of features.

the other deliberation regard to quantitation of nitrite and nitrate in drinking water is the choice of detector. In certain parts of the world, standard IC methods specify UV detection for nitrite and nitrate. One such example is International Organization for Standardization (ISO) 10304-1:2007 Part 1, which targets the determination of bromide, chloride, fluoride, nitrate, nitrite, phosphate, and sulfate in drinking water. Both nitrite and nitrate

absorb in the UV, so one would expect that detection of these two anions should be straightforward using UV detection. One not so obvious nuance is a requirement to detect these anions after the suppression reaction. Several literature references suggest that carbonate eluent absorbs in the UV and that this absorbance introduces excessive baseline noise and interferes with quantitation unless a suppressor is used to convert carbonate to carbonic. In fact, sodium carbonate and sodium bicarbonate are only very weakly absorbing in the ultraviolet. The actual underlying cause of the elevated background and increased baseline noise is because the hydroxide anion absorbs at the low UV wavelengths and hydroxide is always present at appreciable levels when carbonate is present. The suppression reaction removes this hydroxide and thus improves the detection limit when using UV detection with basic eluents.

1.2) Application in pharmaceutical

Chromatography serves as an alternative method of reversed-phase chromatography in the pharmaceutical industry for the determination of drugs including sulpha drugs. this method can be used to help the pharmaceutical industry check the quality of the ingredients. Ingredients range from the chemicals used as active ingredients or excipients to pharmaceutical grade water and water for injectables. IC also can be of use in the analysis of the final formulation. Since the FDA requires drugs to be produced only according to approved procedures, they often confirm authenticity by testing the nature of the excipients or fillers. Since manufacturing processes use distinct excipients such as phosphate or citrate buffers in injectables, or sorbitol, calcium sulfate, or dibasic calcium phosphate as fillers, IC provides a simple approach and is also less time-consuming. it can be used to help with cleaning validation whenever the cleaning solution contains charged species.

1)Extraction procedure: Extract was prepared from the leaves and roots of two years old olive plants with water at room temperature. Internal standard as D-3-O-methylglucopyranose (MeGlu) was used and added inappropriate volume. Extraction was accomplished by

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shaking for 15 min and finally the suspension was centrifuged at 3000 rpm for 10 min. Before the injection the aqueous phase was filtered and passed on a cartridge OnGuard A (Dionex) to remove anion contaminants.
Stationary Phase: Two anion exchange columns Dionex CarboPac PA1 plus a guard column and CarboPac MA1 column with a guard column was used for separation procedure (High Performance Anion Exchange Chromatography).

Eluent: Eluent was comprised of 12 mM NaOH with 1 mM barium acetate. The flow rate was 1mL/min.
Detection: pulsed amperometric detection
Analyte(s): Myo-inositol, galactinol, mannitol, galactose, glucose, fructose, sucrose, raffinose, and stachyose

2) Source: Cochlospermum tinctorium A. Rich.
The extraction procedure: The powdered roots of C. tinctorium were extracted with ethanol (% 96, v/v) using a soxhlet apparatus to remove low molecular weight compounds. Extraction procedures continue until no color could be observed in the ethanol. The residue was extracted with water at 50 ºC, 2 hours two times. The obtained extract was filtered through gauze and Whatman GF/A glass fiber filter and then concentrated at 40 ºC in a vacuum and dialyzed at cut-off 3500 Da to give a 50 ºC crude extract. The extracts were kept at -18

ºC or lyophilized.
Stationary Phase: Anion exchange-DEAE-Sepharose column
Eluent: For obtaining a neutral fraction the column was eluted with water firstly. The acidic fractions were obtained by elution of linear NaCl gradient (0-1.4 M) in water. The carbohydrate elution profile was determined using the phenol-sulphuric acid method. Finally, two-column volumes of a 2 M sodium chloride solution in water were eluted to obtain the most acidic polysaccharide fraction. The relevant fractions based on the carbohydrate profiles were collected, dialyzed, and lyophilized.
Detection: UV detector, 490 nm
Analyte(s): Glucose, galactose, arabinose (in neutral fraction) Uronic acids (Both galacturonic and glucuronic acid), rhamnose, galactose, arabinose, and glucose (in acidic fraction)

1.3) Application in clinical studies
IC is a decisive analytical tool for solving problems in the fields of nephrology, radiology, neurology, oncology, and dentistry. it is used for the determination of
inorganic ions:- fluorides, chlorides, nitrites, nitrates, bromides, phosphates, sulfates, oxalates, cyanides, thiocyanates, thiosulfates, citrates, isocitrates, carbonates. cations:- lithium, sodium, ammonium, potassium, magnesium, and calcium organic substances:-
carbohydrates, peptides, amino acids
In clinical studies, Ic is mostly used for the determination of ions inhuman fluids, such as urine, saliva, and serum. fluids also contain proteins, lipids, metabolites, ions, and excretable drugs. the major application of IC in clinical studies is the determination of NO. Nitric oxide (NO)is a vital messenger in many cellular communication and control systems. Because of the short half-life of NO in aqueous media, the NO assay is performed on its metabolites, i.e. nitrites and nitrates. They are generally regarded as hazardous because they are highly toxic to humans, especially to infants. Their high levels can cause methemoglobinemia and possible death. Nitrites can react with secondary amines and amides and form various carcinogenic N-nitroso compounds. The presence of nitrates in human fluids may be considered as the blood urea-nitrogen indicator linked to the homeostatic balance. IC methods are also used for the determination of total Na, K, Ca, and Mg in the serum, selenium metabolites in urine, triphosphates in intercellular nucleosides, cyanides and sulfides in the blood of the suicide or fire victims, oxycodone and its metabolites in plasma, rodenticidepindone, rodenticide Valone and chlorophacinone in human plasma, thiosulphate in urine, diphacinone in the liver, cyclosporine A in the human cerebrospinal fluid, perchlorate, thiocyanate, and iodide in human milk. It is also employed for people suffering from thyroid disease.

1.4) Application in fruit juice
1)Analysis of organic acid
Organic acids are naturally found in fruit and are important in characterizing the flavor of fruit juice.it is a useful index of authenticity in fruit products since they have lower susceptibility to change during processing and storage than other components of fruits (Camara et al., 1994). Accurate knowledge of organic acid levels (and ratios) might be useful for determining the percentage juice and also for detecting misbranding and/or adulteration in fruit juices since each fruit has a unique pattern of organic acids. The organic acid composition of fruits is also of interest due to its impact on the sensory properties. it is necessary to determine organic acids to assess whether an expensive juice has been illegally adulterated with a cheaper juice. Because organic acid profiles are distinct to each type of fruit juice, evidence of tampering can be evaluated by comparing the known juice fingerprint to that of the suspected adulterated juice. Organic acid profiles can also determine juice freshness or spoilage
This method uses eluent generation to generate high-purity, carbonate-free eluents to suppress baseline drift and therefore improve retention time and integration

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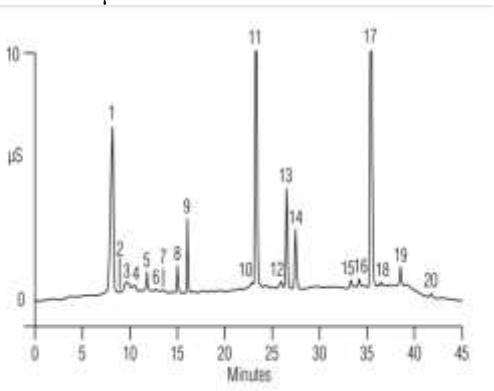
reproducibility. The Dionex IonPac AS11-HC column is the ideal column for this method because its high capacity improves the separation of a wide range of organic acids.

Conditions
Columns: Dionex IonPac AS11-HC Analytical, 4 mm
Dionex IonPac AG11-HC Guard, 4 mm
Eluent: Potassium hydroxide gradient:
1 mM from 0–8 min
1 mM to 30 mM, 8–28 min
30 mM to 60 mM, 28–38 min
Methanol: 10%, 0–38 min
Eluent Source: Dionex EG50 generator
Flow Rate: 1.5 mL/min
Temperature: 30 °C
Detection System: Suppressed conductivity, Dionex ASRS ULTRA suppressor, 4 mm,
AutoSuppression, external water mode
(10 mL/min)
Backpressure: 2900 psi
Background Conductance: 1–4 µS
Injection Volume: 10 µL



Peaks:
1. Quinate 210 mg/L
2. Fluoride <0.1
3. Lactate/Acetate 1 0
4. Glycolate 2.6
5. Formate 3.7
6. Pyruvate 2.1
7. Unknown -
8. Galacturonate 16.9
9. Chloride 2.3
10. Nitrate <0.1
11. Succinate 257
12. Unknown -
13. Sulfate 10.3
14. Oxalate 1 4.8
15. Phosphate 1.8
16. Unknown -
17. Citrate 163
18. Isocitrate 1.0
19. Trans-aconitate 2.7
20. Unknown -

Analysis of carbohydrate
Rapid and cost-effective methods of carbohydrate determination in fruit juice are of high importance since, unscrupulous companies, manufacturers seek substantial benefits using adultered juice to gain market advantages over honest competitors, using cheaper ingredients (fruit juice, sugar, and syrups) and falls label indication for the consumer.
HPAE coupled with PAD is a well-established technique to identify and quantify carbohydrates in food and beverage samples. This method provides key metrics of product quality and related properties, contamination, and adulteration. HPAE-PAD allows direct quantification of nonderivatized carbohydrates with minimal sample preparation and resolves most carbohydrates from sugar alcohols and organic acids, while not detecting sodium chloride commonly present in fruit juices. . Capillary Reagent-Free™ ion chromatography (RFIC™) systems expand the application of IC to carbohydrate analysis for the food and beverage industries by bringing enhanced mass sensitivity, ease-of-use, and reproducibility to the routine determination of carbohydrate.

Conditions
Column: Dionex CarboPac PA 20, 0.4 × 150 mm

Eluent: 50 mM potassium hydroxide (EG)
Flow Rate: 10 µL/min
Inj. Volume: 0.40 µL
Detection: PAD, 4–potential carbohydrate, Au
Ref. Electrode: PdH

Gasket Thickness: 25 µm

Samples: Juice samples
(5000× dilution)
Standard (20 µM)
temperature 30 ºC

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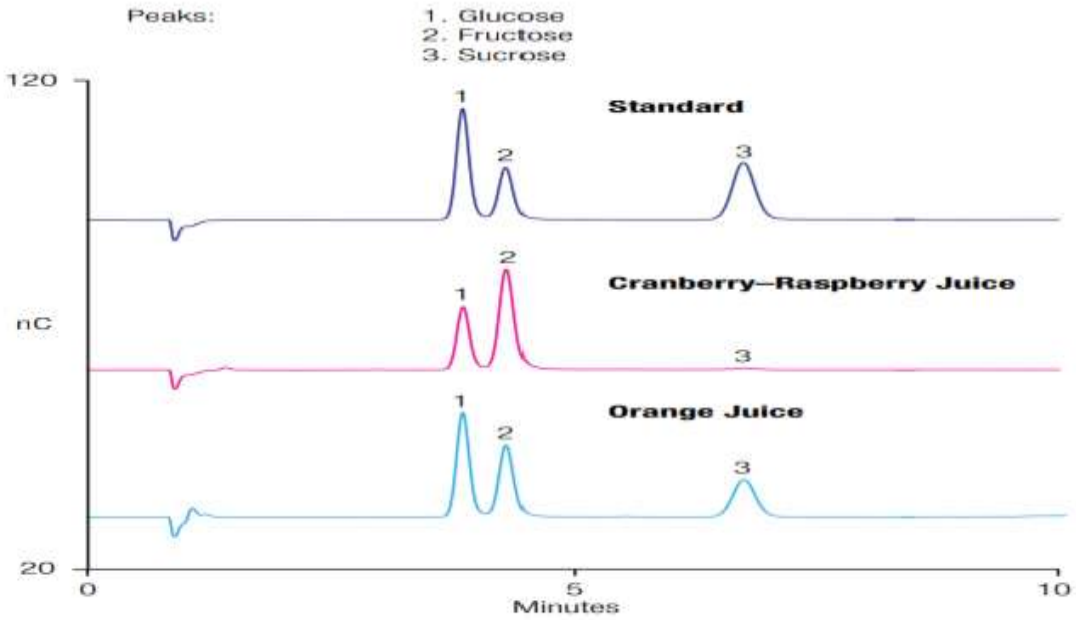

Analysis of juices for carbohydrates by capillary HPAE-PAD.

1.5)Application in beer

The compounds of interest for the beer industry range—from inorganic ions, organic acids, and hop bittering principles that contribute to the overall taste and bitterness of the beverage—to proteins, carbohydrates, and alcohols that are monitored to determine the extent of fermentation therfore, analytical monitoring of the beer is essential to guarentee quality and meet consumer ptoduct. The fiished beer product may also be analyzed to determine the concentration of added preservatives and colorants.

This application note describes the use of ion-exchange or ion-exclusion chromatography for the determination of fie classes of compounds of interest to the brewing industry, including: carbohydrates, alcohols, organic acids, inorganic anions, and inorganic cations. One of two forms of detection is used, pulsed amperometry or conductivity detection.

Conditions

Column: Dionex CarboPac PA1 (4 x 250 mm)

Eluent 1: Deionized water

Eluent 2: 500 mM Sodium hydroxide

Gradient:

Time	**E1**	**E2**	**Comments**
Initial	99	1	Reequilibrate
5.00	99	1	Inject
6.00	99	1	Back to Load
20.00	91	9	
45.00	0	100	
50.00	0	100	

Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL

Detection: Pulsed amperometry, gold electrode

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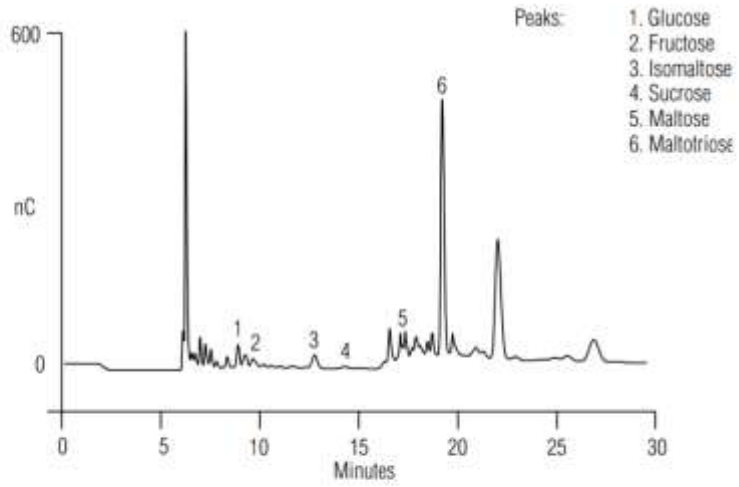

Separation of mono-, di-, and trisaccharides in an American beer by ion-exchange chromatography with pulsed amperometric detection. The sample was diluted 1:10 before injection.

1.6) Application in food industrie

In recent years, awareness of food quality and ingredients has grown substantially. Consumers want to know what they are eating and expect thorough and detailed food labeling to track this. many many foodstuff have complex matrix because of which it is challanging to analysi foodstuff. Ion chromatography (IC) can address many of these challenges better than more traditional methods .

Nitrite and nitrate salts are use as preservative foe meat and meat product. they are labbeled on food as E249-E252. these salt prevent the bacterial growth keep the meats red colour and enhance the flavour. nitrate salt have less toxicity however long term exposer is concern, as nitrate is reduce to nitrite in lower gut and nitrite is a precourser of nitrosamine, which are classified as carcinogenic.

Samples And Sample Preparation

- Meat, sausages, beverages, vegetables (5 g homogenized)
-Carrez precipitation
- Diluted 100-fold, filtered

MISP

- Inline Ultrafiltration

Experimental

Analytes were separated by isocratic anion exchange on two columns in series, followed by
sequential suppression and UV/VIS detection. The two columns with different properties were used in series to avoid coelution of niytrite with other components. Atemperature of 52 ºC further improved the resolution of nitrite.

II. RESULT:

The calibaration range for nitrite was o.o2-2.00mg/l and for nitrate 0.05-5.00mg/l. a wide variety of food and beverage sample were evaluated, showing high reproduciblity of concentration valves, and negligible interference from matrix compounds. limit of quantification were well below 5mg/kg for sodium nitrite and sodium nitrate in all tested.

Metrosep A Supp 7 - 250/4.0 +
Metrosep A Supp 5 - 50/4.0
Eluent 3.6 mmol/L Na_2CO_3
+ 15% methanol

Flow 0.7 mL/min
Temp 52 ºC
Injection 50 µL
Detection UV 205 nm

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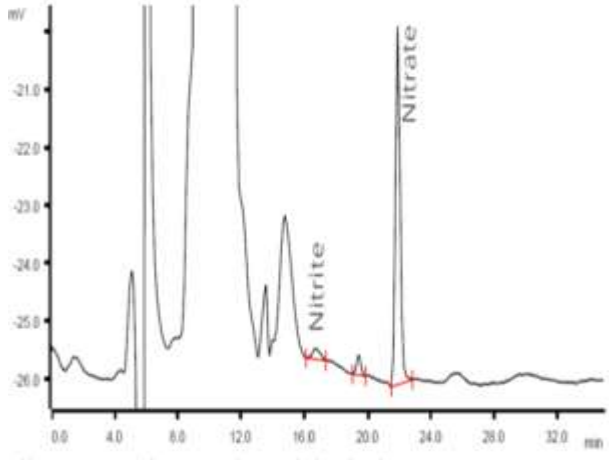

III. CONCLUSION :

Ion chromatography is a developed, less time-consuming, and powerful technique for the multicomponent analysis of many ions and substances in many fields. there are many advantages of using ion chromatography 1)many detection applications 2)low cost accurate and precise result3)high selectivity and separation efficiency. presently IC needs a meager amount or concentration of sample for analysis but as the technique of IC getting advance it may result in futer less concentration is needed for the analysis of complex.IC is mostly used everywhere and its application is increasing.

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Nitrite and nitrate salts are used as preservatives for meat and meat