Determination of Ions F⁻, Cl⁻, NO₃⁻, SO₄²⁻, PO₄³⁻ In The Blood Serum of Women With Breast Cancer Using Ion Chromatography Techniques

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Abstract

By using ion chromatography technique for determining the concentration ions (Floride, Chloride, Nitrate, Phosphate and Sulphate) in blood serum was achieved for women infected with breast cancer in groups of different year range (16-50) year and collaboration with Ministry of Health

The nature of the disease is not regularity in the growth and cell proliferation in breast tissue of women and the study of anion ions because of their importance in giving to the occurrence of the injury early in the growing signs of sensing or decrease the concentration of ions in the blood serum of women.

For operational conditions for ion chromatography to better separate and choice of mobile phase and install the variation of the flow rate and the retention time ions for determining ions in standard solution prepared containing different concentrations ion for an individual in mixture and obtained a calibration curve to calculate the slope, correlation coefficient R^2 between(0.9998-0.9999) and RSD that was in less than 1%.

To extraction the anions by mix solution NaOH: ACN for blood serum with centerfuges and determined that anion in ion chromatography, for a comparison of this work with different analysis method Universally adopted in ASTM method references as UV-Visb spectrophotometry, ion selective electrode (ISE) and turbidity.

Keywords: breast cancer, Ion Chromatography, anions, blood serum

Introduction

Breast cancer is one of the cancers that have spread in recent times, especially in the hot area of the world and the chaos of home use of materials, chemicals, wars around the world and lack of health education of the people in the developing world.

This disease and feels like other cancers in infected cells growing in tissue growth and large division in the cells is cut or malignant cancers tumours or non malignant.

The Shintani and Ubs (1985) may determine the cation and anion in blood serum samples and urine using ion chromatography with conductivity cell and ultraviolet cell , were found that there are concentration of ammonium ions in urine samples is no difference between this analytical method and colour method.

Sakuma and Kitamura (1987) has calculated RSD for anion is 1.5% as loss by using ion chromatography and ultraviolet detector at a λmax 595 NM, But Salas and Colmonarez(1995) using ion chromatography with sodium phosphate buffer as a mobile phase and detector UV in λmax 284 nm.

For study the best mobile phase that leads to good separation for anion the Benzo and Salas (2002) using sodium hydroxide as a separation solution in two concentrations (6M,12M) to separating some ions (chloride, nitrate, Bromide, phosphate and sulphate) and the result for then were good in blood serum samples.

Determination Trichloroacetate in human blood serum and urine by Itoh (1989) used an ion chromatography technique, Miyanchi and Takahashi (2010) were found that measure urea by a used conductivity detector in limit test 0.2 mg/L, effectively 190 mg/L and calibration curve 0.8-25 mg/L.

The extraction the ions from blood serum and precipitate of protein, there are many Scientists who have developed separation methods, including Jebrail and Wheeler (2009) by used mix of solvent CCL_4 and CAN as a ratio (70/30 v/v) and Feraz and Fluza (2004) used mix methanol and CCL_4, but Garther and Witkamp (2005) there extract chloride and sulphate ions by using a mix (50-100%) salt-ethylene glycol.

Instrument part

A. A device used:
   1. Ion chromatography Metrohm 881 Compact
   2. Potentiometric Orion 940
   3. Turbidity HACH
   4. Specrophotometic UV-Vis -Shimadzu –Japan 1650
   5. Balance mettle AE163
   6. Centrifuge
B. Ion selective electrode Chloride,Nitrate and Floride
C. Chemicals used highly pure from Fluka and BDH Company
D. Separation method:
   1. Treatment of 0.5 ml of serum with 0.05 ml (2M NaOH) and 0.15ml of deionized water and stirring , Add 1 ml of ACN (Acetonitrile) and mixing and separation by centrifuge for 5 min. in 755xg and pull 1 ml of liquid to 5 ml of deionized water, dilute 1:10 of blood serum samples and used a special filter (Benzo 2002)
   2. Treatment of 0.2 ml of serum with ACN and mix by centrifuge 7 min. , then pull 0.1 ml of solution in 1 ml deionized water.

Result and Discussion

By used on several types of mobile phase in operation conditions for ion chromatography for determining the concentration anion ions to be measured in serum womens blood voluntarily and infected serum of breast and non-inflected .

For selected the number of ions as chloride , floride, nitrate,phosphate and sulphate with low concentration of these ions are important a promotion of remote sensing for breast cancer in women.

The attempt to get a good amount of space in the figure chromatograph in serum of ions measured and interferences between anion ions chosen for determination was the experience of a number of solutions prepared mobile phases of salts.

In used sodium Hydroxide, NaOH in different molarity (6M,12M) on mobile phase and sodium carbonate Na_2CO_3 (9M) in determination anion ions on standard solutions for the preparation that attended the standard salt chloride , floride, nitrate,phosphate and sulphate with range concentration (1-20 mg/L)

These results were disappointing because of interference between the conductivity peaks of ions in the mobile phase and the presence of retention time of this anion Because the concentration of anion is low was using a mixture of sodium salt (carbonate and bicarbonate) as 1.47mM each one Preparation some the standard solution for ionic salts in standard mixture with range concentration (1-20 mg/L) and determined this mixture in Fig(1) for concentration (10 mg/L) for each ions were found through a form that figure chromatograph is clear and not interferences between the tops of the ions and good separation in the mixture for ion, table (1) shows the ion chromatography condition .

Measured lotions standard prepared for chloride , floride, nitrate,phosphate and sulphate in concentration range (1-20 mg/L) and through the data has been installed at the retention time for each ions and also in the table (2), and calibration curve for this concentration as shown in figure (2).

Table (3) shown the calculated correlation coefficient R^2 and extracted linear equation for each calibration curve .

For comparsion of this analytical technique with some analytical techniques are found in ASTM (1980) reference for example, ion selective electrode (ISE) to determine concentration chloride and floride ions , spectrophotometric UV for determining phosphate (ammonium meta vinadate method) and a turbidity method to determine sulphate ions .

Table (4) shows determination anion concentration in prepared standard solution in different concentration for analytical techniques and calibration curve of this anion ions and R^2 , and calculated RSD and recovery in mixture anion ions and shown in table (5).

When a withdrawal of blood samples from volunteers and across groups ranging number of inflected disease volunteers or non – inflected in each group (25) women and treatment this sample by separation method for determination by ion chromatography and recorder that data for each ion , shown in table (6).

No interferences with this anion ion concentration and found places for concentration chloride ions in samples the blood serum women inflected but more anion ions are accepted within the global border
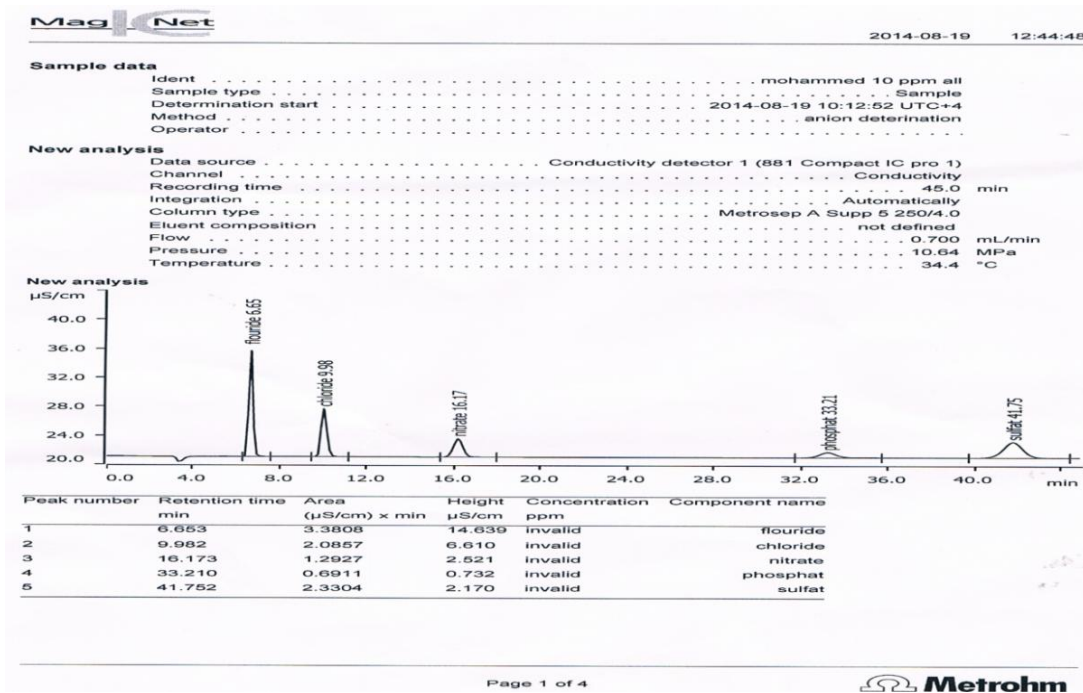
Figure 1: chromatograp 10 mg/L of the mixture ion F,Cl,NO$_3$,PO$_4$ and SO$_4$ by ion chromatography
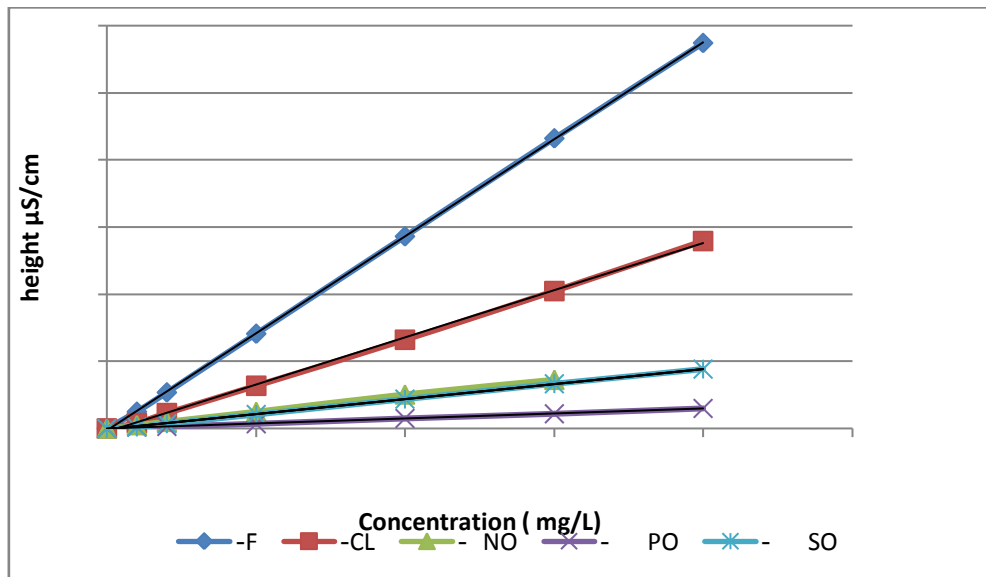

Figure 2: Calibration curve for different concentration of F$^-$, Cl$^-$, NO$_3$- , PO$_4^{3-}$, SO$_4^{2-}$ by ion chromatograpy

Table 1: ion chromatographic conditions

Eluent	Flow rate	Pressure	Column heater	Column
1.74 mM sodium carbonate + 1.74 mM sodium bicarbonate	0.7 mL/min.	10.1 Mpa	33.2 C	Metrosep A Supp 5 250\4.0

Table 2: Retention time for each ions in ion chromatography

No.	Anion	Retention time min.
1.	F^-	6.653
2.	Cl^-	9.982
3.	NO_3^-	16.173
4.	PO_4^{3-}	33.21
5.	SO_4^{2-}	41.752

Table 3: Calculation of straight line equation and correlation coefficient

No.	Ions	Equation	Correlation Coefficient R^2
1	F^-	Y=1.4433x-0.1278	1.0000
2	Cl^-	Y=0.7032x-0.2507	0.9992
3	NO_3-	Y=0.2253x-0.077	0.9995
4	PO_4^{3-}	Y=0.0753x-0.0153	0.9994
5	SO_4^{2-}	Y=0.2253x-0.077	0.9995

Table 4: determination concentration different ions with different techniques and calculated correlation coefficient

Anion	Conc. range mg/L	Method	R^2	Slope
F	1-100	ISE	0.9999	59 mV\decade
Cl	1-100	ISE	0.9990	57 mV\decade
PO_4	1-20	UV-Visb	0.9998	----
SO_4	1-20	Turbidity	0.9996	-----

Table 5: shows the calculation of recovery and RSD for anion in different techniques

Anion	Method	Recovery%	RSD%
F^-	ISE	88	0.43
Cl^-	ISE	85	0.46
PO_4^{3-}	UV-Vis	90	0.40
SO_4^{2-}	Turbidity	79.5	0.53

Table 6: Determination of concentration ions mg/L by using ion chromatography in serum samples

N0.	Number women	Sample	CL^- mg/L	F^- mg/L	PO_4^{3-} mg/L	SO_4^{2-} mg/L
1	25	Control(16-25)year	150	6	3	0.72
2	25	Control(25-50)year	160	7	5	1
3	25	16-25 year	200	10	8	4
4	25	25-50 year	220	12	8	5
5		المعدل الطبيعي في الدم	95-107	5	3-4.5	0.7

Reference

[1]. Breast Cancer and The Environment (2012). The National Academies press, Washington. D. C ,USA.

[2]. Shintani. H, Ube. S, (1985) Simultaneous determination of Serum cations, anions and uremic toxins by ion chromatography using immobilized enzyme. J.Chromatographic. 344;145-156.

[3]. R.Sakuma,T.Nishina and M. Kitamura, (1987)Deproteinizng methods Evaluated for the determination of uric acid in serum by reversed- phase liquid chromatography with ultraviolet detection. J. Clin. Chem, 33/8, 1427-1430

[4]. R. Salas_ Auvert, J. Colmenarez, H. Ledo, M. Colina, E. Gutierrez, A. Beavo, L. Soto and S.Azuero (1995). Determination of anion in human and animal tear fluid and blood serum by ion Chromatography; J. Chromatography A,706 (1-2): 183-189

[5]. Benzo,Escalona,Salas,Gomez,Quintel.Marcano,Ruiz,A.Garabotoand and Bartoli (2002) .Evaluation of select variables in the ion chromatographic science. Determination of F^-, Cl^-, NO_3,SO_4^{2-} and PO_4^{3-} in serum .J.Chromatographic Science .40,101-105

[6]. H. Itoh (1989). Determination of Trichloro acetic in human serum and urine by ion chromatography; J. Analyst, 114:1637-1640

[7]. Miyauchi T,Miyachi. Y, Takahashi, M, Ishikawa. N and Mori. H. (2010). Determination of urea in serum based on the combination of an enzymatic reaction with immobilized urease and ion chromatographic analysis. J. Anal., Sci., 26(8): 847-851

[8]. Mais. J. Jebrail and Aaron R. Wheeler (2009). Digital Microfluidic Method for protein extraction by precipitation. J. Anal. Chem. 81, 330-335.

[9]. Ferraz, M. Fiuza, Dos Santos, Pontes de Carvalho and Soares (2004). Comparison of six methods for the extraction of lipids from serum in terms of effectiveness and protein preservation. J. Biochem. Biophys. Methods 58, 187-193

[10]. Robert. S. Gutner and Geert -Jan Witkamp (2005). Regeneration of mixed solvent by ion exchange resin : Selective removal of chloride and sulfate. J. Seperation Science and Technology, 40, 12, 2391-2410

[11]. Annual book of ASTM standardy (1986) Water and Environmental Technology (section 11) D512, D4127, D11