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Journal of Pharmaceutical and Medicinal Chemistry

Volume 7 Number 1
January – June 2021

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Original Article

Journal of Pharmaceutical and Medicinal Chemistry
Volume 7 Number 1, January – June 2021
DOI: http://dx.doi.org/10.21088/jpmc.2395.6615.7121.1

A Comparative Validation Study for the Estimation of Cefotaxime Sodium by Spectrophotometric and Fluorimetric Methods

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How to cite this article:
Aswathy S R, K S G Arul Kumaran, Devi Swapna P V, Anjali Sruthi S, A Comparative Validation Study for the Estimation of Cefotaxime Sodium by Spectrophotometric and Fluorimetric Methods. J Pharmaceut Med Chem. 2021;7(1):9-15.

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Abstract

In present research, a comparative validation study for the estimation of cefotaxime sodium in pharmaceutical formulations by spectrophotometric and fluorimetric methods were performed.

The developed methods depend on the determination of cefotaxime sodium by using 2, 4-Dinitro phenyl hydrazine. By using 2, 4-Dinitro phenyl hydrazine, these drug form ion-pair complex in acidic medium. This coloured complex shows maximum absorbance at 408nm by spectrophotometric method (Method A) and fluorescent intensity was measured by using fluorimetric method (Method B).Linearity calibration curve were obtained in a concentration range of 1.0-6.0 µg/ml for cefotaxime sodium by using both of these methods. The result of analysis of these methods shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation.

The developed methods were validated for various parameters as per ICH guidelines like Accuracy, Precision, Linearity, and range, LOD and LOQ, Ruggedness and Robustness. %RSD will be less than 2 for all the validation parameters. Recoveries studies revealed that results within the specified limits. Hence the proposed spectrophotometric and fluorimetric methods were found to be satisfactory and could be used for the routine analysis of cefotaxime sodium in their marketed formulations. Statistical comparison of the results with the reference methods showed excellent agreement and proved that no significant difference in the accuracy and precision.

Keywords: Cefotaxime sodium; 2, 4-Dinitrophenyl hydrazine; Fluorimeter and UV-Visible Spectrophotometer.

Introduction

Cefotaxime sodium (Fig. 1) is a third-generation cephalosporin antibiotic. Like other third generation cephalosporins, it has broad spectrum activity against Gram-positive and Gram-negative bacteria. Cefotaxime injection is used to treat certain infections caused by bacteria including pneumonia and other lower respiratory tract (lung) infections, gonorrhoea, meningitis and other brain spinal cord infections.[1-3]

In literature survey several analytical methods like HPLC[4-13] and spectrophotometric[14-19] immunoassay for screening, fluorimetry and Mass spectrometry[20-25] methods have been reported on the determination of cefotaxime sodium in combination with other drugs. The purpose of this study was to develop and validate a simple analytical method to quantify cefotaxime sodium in injectables, using spectrophotometry and fluorimetric methods. The methods have been successfully applied to the determination of the cited drugs in their commercial pharmaceutical formulations. The results obtained by these methods were statistically compared.

Materials and Methods

Drugs and Formulations

The reference samples of Cefotaxime Sodium (API) were provided from Lupin Pharmaceuticals, Mumbai. The commercial formulation was procured from the local market.

Chemicals and Solvents

2,4-Dinitro Phenyl Hydrazine (1% w/v), ethanol and dilute H_2SO_4 were used for these studies. Freshly prepared double distilled water was used throughout the experiment.

Instrumentation

Systronics-2203 UV Visible double beam spectrophotometer (10mm optical path length matched quartz cell,50 nm min^{-1} scan speed) and 5 nm fixed slit width) was used for recording spectra and absorbance measurements; Elico fluorimeter with a xenon discharge lamp, excitation and emission slit of 10 nm and quartz cuvettes of1x1 cm was used for emission studies. Other instruments like pH-Meter (MKV1, Systronics) and Digital Balance are used for this study.

Preparation of Standard Solution

100 mg of cefotaxime sodium was weighed and transferred in to 100 ml volumetric flask. The drug was dissolved and the volume was made up to the mark with water to obtain final concentration of 1000µg/ml (Stock -A solution).

Preparation of working standard solution

From the stock-A solution, 1 ml was pipette out and transferred in 100 ml volumetric flask and add 10 ml of 2, 4-DNP and 5ml dilute sulphuric acid then the volume was made up to the mark with water to obtain the final concentration of 10µg/ml (stock –B solution).

Results and Discussions

The main step in the development of an analytical method is to improve the conditions and parameters which should be followed in the development and validation. Different solvents were studied (methanol, ethanol, acetone and water), the criteria employed were the sensitivity of the method and availability of the solvent. From a solvent effect studies and spectral behaviours of Cefotaxime sodium, water was selected as solvents for the suggested spectrophotometric and fluorimetric methods. The method sensitivity such as LOD and LOQ and analytical parameters such as correlation coefficient, intercept and slope of the calibration equations was tested.

Method Development

In this method, based on spectrophotometric and fluorimetric determination of cefotaxime sodium in pure and dosage forms were developed by using 2, 4-Dinitro Phenyl Hydrazine in dilute sulfuric acid. The analytical conditions were selected, keeping mind that the chemical structure of Cefotaxime sodium.

Spectrophotometric method (Method-A)

Cefotaxime sodium react with 2, 4-DNP in dilute sulfuric acid (pH-3.2) to get a yellow-coloured ion-pair complex and measured the maximum absorbance at 408nm (Fig. 1). The ion-pair formation is due to Cefotaxime sodium contains carbonyl keto group react with 2, 4-DNP n acidic medium. So, 2,4-Dinitro Phenyl Hydrazine was converted to 2, 4-Dinitro Phenyl Hydrazones give yellow coloured ion pair complex, which is quantitatively determined by spectrophotometric methods.

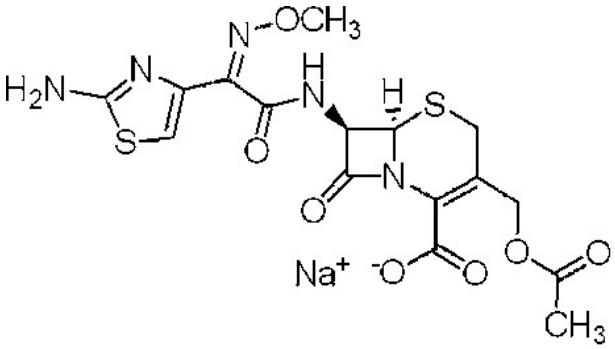

Fig. 1: Structure of Cefotaxime Sodium.

Fuorimetric method (Method-B)

Cefotaxime sodium react with 2, 4-DNP in dil. H_2SO_4 (pH-3.2). Yellow colored fluorescent compound is formed and measure the fluorescent intensity. This was formed due to Cefotaxime sodium contains carbonyl keto group react with 2,4-DNP in acidic medium. 2,4-Dinitro Phenyl Hydrazine was converted to 2,4-Dinitro Phenyl Hydrazones give yellow colored ion pair complex, which is quantitatively determined fluorimetrically.

Analysis of Cefotaxime sodium

Quantitative analysis of Cefotaxime sodium content (Claimed concentration 100 mg/1ml) in commercially available injection by UV and fluorimetric methods. The assay procedure was repeated for 6 times, mean weight of standard drugs, of sample were taken and calculated. Prepare 5 µg/ml of standard and sample solution were also prepared and assayed for content of cefotaxime sodium against the reference standard. The content of cefotaxime sodium in the marketed brands was determined, the percentages of individual drugs found in formulations, amount and relative standard deviation in formulations were calculated. The result of analysis shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation Table1.

Table 1: Estimation of cefotaxime Na.

Factors (n = 5)	UV-Visible method		Fluorimetric method	
	Brand A	Brand B	Brand A	Brand B
Mean (%w/v)	99.0%	99.7%	98.5%	98.4%
Labelled amount (mg)	0.01	0.01	0.01	0.01
Amount found (mg)	0.00985	0.0099	0.0099	0.00994
SD	0.00008	0.00004	0.00010	0.00008
R.S.D (%)	0.804	0.402	1.02	0.808

Method Validation

A) *Accuracy*

The recovery studies (Table 2 &3) were carried out 3

times at 50%, 100% and 150% levels and the percentage recovery and percentage relative standard deviation of the percentage recovery were calculated. Recoveries studies revealed that results within the specified limits and the method were found to be accurate.

Table 2: Recovery Studies for Cefotaxime Na.

Level of addition (% pure drug)	Con. of drug in formulations (µg/ml)		Conc. of pure drug (µg/ml)		Total conc. of drug found (µg/ml)	
	Method A	Method B	Method A	Method B	Method A	Method B
50%	5	5	2.	2.5	7.47 7.48 7.47	7.46 7.47 7.46
100%	5	5	5	5	10.07 10.05 9.98	10.01 10.09 9.95
150%	5	5	7.5	7.5	12.44 12.45 12.44	12.43 12.45 12.43

Table 3: Statistical validation data for accuracy determination of Cefotaxime Na.

Level of Recovery %	Mean		Standard Deviation		%RSD		% Analytical recovery	
	A	B	A	B	A	B	A	B
50%	7.4733	7.46	0.0057	0.0057	0.076	0.07	99.64	99.6
100%	10.17	10.01	0.0041	0.412	0.040	0.409	101.4	101.7
150%	12.44	12.43	0.0016	0.0019	0.012	0.014	99.94	99.52

B) *Precision*

The intra-day and inter-day precision studies of the developed method (Table 4 & 5) confirmed adequate sample stability and method reliability, where all the RSDs were less than 2%.

Table 4: Precision studies for cefotaxime Na-Method A.

Intraday (n=6)				Interday (n=6)			
Conc. (µg/ml)	Ab.(1)	Ab.(2)	Ab.(3)	Conc. (µg/ml)	Ab. (D-1)	Ab. (D-2)	Ab. (D-3)
5	0.695	0.695	0.695	5	0.695	0.695	0.695
5	0.696	0.696	0.694	5	0.696	0.694	0.694
5	0.695	0.694	0.695	5	0.695	0.695	0.695
5	0.696	0.695	0.694	5	0.696	0.696	0.696
5	0.695	0.696	0.696	5	0.695	0.695	0.695
5	0.695	0.695	0.695	5	0.696	0.696	0.694
MEAN	0.6953	0.6951	0.6948	MEAN	0.6955	0.6951	0.6948
S.D	0.000517	0.00075	0.000753	S.D	0.000547	0.00075	0.000753
%RSD	0.0743	0.1078	0.1084	%RSD	0.0786	0.1078	0.1079

Table 5: Precision studies for cefotaxime Na-Method B.

Intraday (n=6)				Interday (n=6)			
Conc. (µg/ml)	**Ab.(1)**	**Ab.(2)**	**Ab.(3)**	**Conc. (µg/ml)**	**Ab. (D-1)**	**Ab. (D-2)**	**Ab. (D-3)**
4	66	66	66	4	66	66	67
4	67	67	67	4	67	67	66
4	66	66	66	4	66	67	67
4	66	66	67	4	67	67	66
4	67	67	67	4	66	67	67
4	66	67	67	4	66	67	66
MEAN	66.33	66.5	66.66	MEAN	66.33	66.83	66.5
S.D	0.5164	0.5477	0.5164	S.D	0.5164	0.4082	0.5477
%RSD	0.7785	0.8236	0.7746	%RSD	0.7785	0.6108	0.8236

C) *Linearity and range*

For linearity six points calibration curve were obtained by both method A and B in a concentration range 1.0-6.0 µg/ml. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for cefotaxime Na. are shown in (Fig. 3 & 4). Linearity results both Method A and B were depicted in Table 6.

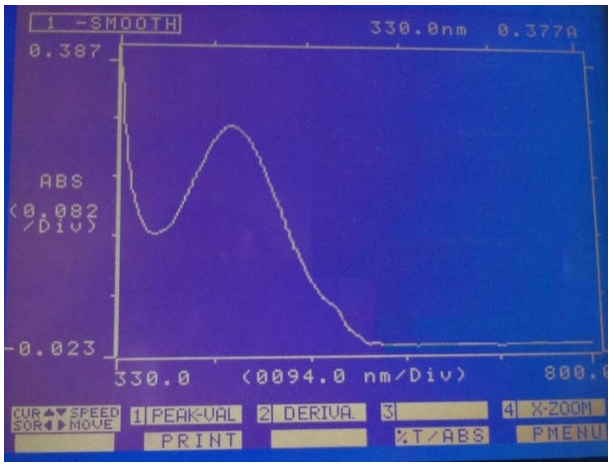

Fig. 2: Spectrum of Cefotaxime sodium using 2, 4-DNP.

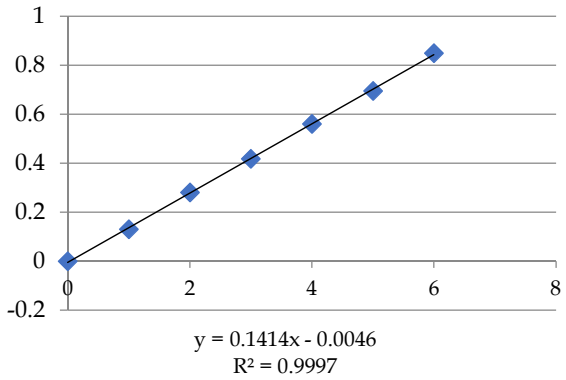

Fig. 3: Calibration curve of Cefotaxime sodium (Method A).

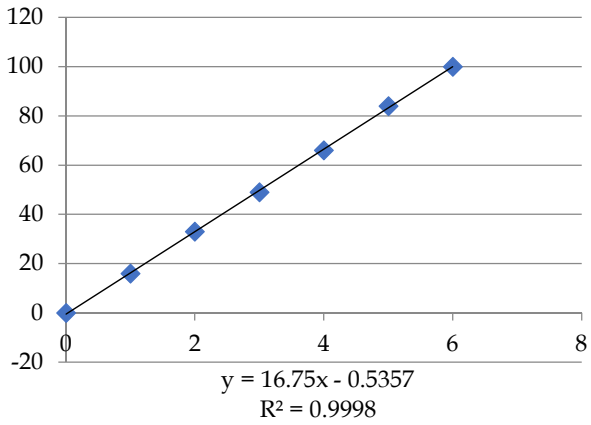

Fig. 4: Calibration curve of Cefotaxime sodium (Method B).

Table 6: Overview of the linearity data for cefotaxime sodium by the UV Visible spectrophotometric and Fluorimetric methods.

Parameters	UV-Visible method	Fluorimetric method
λmax	408nm	-
Regression Coefficient (r^2)	0.9997	0.9998
Slope	0.1414	16.75
Intercept	0.0046	0.5357
Standard Deviation	0.000517	0.6575
Concentration Range (µg mL^{-1})	1.0-6.0	1.0-6.0
Number of Points	6	6

D) *Limit of Detection (LOD) & Limit of Quantification (LOQ)*

The limit of detection and limit of quantification were calculated from calibration curve and LOD and LOQ of Cefotaxime sodium in is given in Table 7.

Table 7: Limit of Detection (LOD) & Limit of Quantification (LOQ) for Cefotaxime Na.

Method	LOD µg/mL	LOQ µg/mL
Method-A	0.141	0.036
Method-B	0.129	0.392

E) *Ruggedness*

The ruggedness of the methods was demonstrated by conducting the experiment on two different analyst and %RSD was calculated by Method A and B shown in Table 8. Variation in percentage content was found to be within the limit so the method is rugged in nature.

Table 8: Ruggedness of Cefotaxime Na.

SL NO:	Conc.(µg/ml)		Method-A		Method-B	
			Analyst-1	Analyst-2	Analyst-1	Analyst-2
	A	B	Absorbance	Absorbance	Absorbance	Absorbance
1	5	4	0.695	0.695	66	66
2			0.696	0.694	65	66
3			0.695	0.695	66	65
4			0.696	0.694	65	67
5			0.695	0.696	66	66
6			0.695	0.695	66	66
Ab. Mean			0.6953	0.6948	65.66	65.833
S.D			0.000517	0.000753	0.5164	0.6575
%RSD			0.0743	0.1084	0.7864	0.9987

F) *Robustness*

The result of robustness study of the developed assay method was established in Table 9. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. Hence the analytical method would be concluded as robust.

Table 9: Robustness of Cefotaxime Na.

SL NO:	Conc.(µg/ml)		Method-A		Method-B	
			Room Temp.	AC. Temp.	Room Temp.	AC. Temp.
	Method A	Method B	Absorbance	Absorbance	Absorbance	Absorbance
1	5	4	0.678	0.671	0.684	0.683
2			0.671	0.670	0.684	0.683
3			0.673	0.669	0.681	0.683
4			0.675	0.672	0.686	0.684
5			0.674	0.673	0.684	0.684
6			0.676	0.674	0.684	0.684
Ab. Mean			0.674	0.671	0.6838	0.6835
S.D			0.0022	0.0035	0.0096	0.0045
%RSD			0.326	0.521	1.40	0.658

Comparative Study

The two methods were successfully applied for the determination of the studied drugs in injections. Statistical comparisons between the results obtained by the suggested fluorimetric and UV-Visible spectrophotometric methods of analysis of the cited drugs were carried out, and no significant difference between them.

A linear relationship was found between cefotaxime sodium concentration and response time of both UV and Fluorimetric methods. The precision data obtained for the two methods are tabulated in Table 4 and 5. Table 6 shows regression analysis data for both the methods. Both UV and flourimetric method shows the regression coefficient of 0.999. Both UV-visible spectrophotometric and fluorimetric methods showed R.S.D. values lower than 2% presenting good precision, however UV Visible spectrophotometric method was more precise than florimetric method. Accuracy was investigated by means of % recovery experiments (n=3) using developed methods. Both spectrophotometric and flourimetric methods exhibited recoveries close to 100%, however better recovery was achieved by UV Visible-method. The LOD and LOQ for UV Visible method were found to be

0.141 µg mL-1 and 0.036 µg mL-1, respectively. Forfluorimetric method, LOD and LOQ were found to be 0.129 µg mL-1 and 0.392 µg mL -1, respectively.

Conclusion

This study showed a possibility to use 2, 4-DNP as a reagent for the spectrophotometric and flourimetric methods determination of cefotaxime sodium. The developed method was validated for various parameters as per ICH guidelines like Accuracy, Precision, Linearity and range, LOD and LOQ, Ruggedness, Robustness. The results obtained were within the acceptance criteria for the parameter. Hence it can be concluded that, the proposed methods (both UV Visible Spectrophotometric and fluorimetric methods) were found to be satisfactory and used for the routine analysis of cefotaxime sodium in their marketed formulation. However, the UV- Visible spectroscopic method is showed to be more sensitive, accurate and precise than fluorimertic method.

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Review Article

Journal of Pharmaceutical and Medicinal Chemistry
Volume 7 Number 1, January – June 2021
DOI: http://dx.doi.org/10.21088/jpmc.2395.6615.7121.2

Pharmaceutical Analysis of Febuxostat: A Review

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How to cite this article:
Asif Husain, Mohd Umar Haris, Mustafa, Nawazish Sajjad, et. al., Pharmaceutical Analysis of Febuxostat: A Review. J Pharmaceut Med Chem. 2021;7(1):17-21.

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Abstract

Febuxostat is an orally active potent xanthine-oxidase inhibitor which inhibits the formation of uric acid thereby preventing gout. Gout is a severe chronic inflammatory disorder if not treated properly; it leads to permanent damage of body tissues and joints. Febuxostat is a well-tolerated and safe antihyperuricaemic agent that is effective to manage and treat chronic conditions of gout. Different forms are available in the market, therefore analysis of febuxostat is an important and inseparable part of industries involved in febuxostat preparations. This review covers important properties, and different methods of analysis of Febuxostat viz. UV, HPLC, UPLC, HPTLC, LC-MS, X-ray, and spectrophotometry.

Keywords: Gout; Assay; Chromatography; HPLC; Spectrophotometry; X-ray.

Introduction

Gout, a type of inflammatory-arthritis, is associated with the formation of monosodium crystals in the joints which causes tissue damage. Gout is characterised by repeated episodes of red, tender, hot and swollen joint. The pain of arthritis comes rapidly and in less than a day gets intense. In maximum cases, joints of big toe get affected and this condition may lead to tophi, kidney stones or kidney damage.[1-4]

Allopurinol as a drug therapy is used widely in gout patients which acts as urate lowering agent. Initially it is given at low dose of 100 mg and then the dose is increased as per condition of the patients. Side effects associated with allorpurinol are: rashes, fever, renal function deterioration, hypersensitivity syndrome. Due to many side effects associated with existing drugs there have been urgent requirements for potential drug(s) with least or no side effects for successful gout management. [5,6]

Febuxostat (Fig. 1) is a new oral xanthine oxidase (non-purine) inhibitor available in the market to treat chronic hyperuricaemia and gout. It appears to be an effective drug for gout as it significantly lowers the serum urate levels. Potency of febuxostat is 10-30 times than that of allopurinol. It is structurally different from allorpurinol and lacks purine ring. Febuxostat, taken orally10-300 mg, shows a half-life of 5-8h. It bounds completely to plasma protein with 99% binding.[7-9]

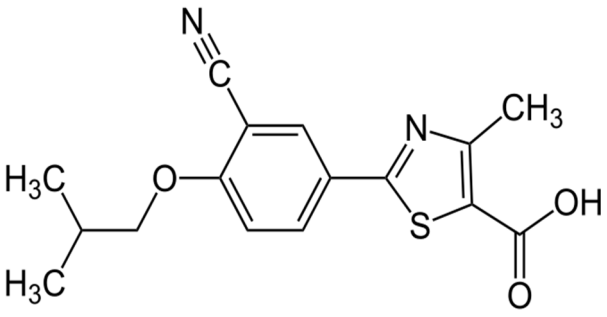

Fig. 1: Structure of Febuxostat.

Febuxostat is metabolised in liver by uridine diphosphate glucuronosyl transferase system and oxidized by cytochrome P450 system and its metabolites get excreted in urine and faeces. Febuxostat is also co-administered with other drugs such as colchicine, certain NSAIDs

(naproxen andindomethacin), warfarin hydrochlorothiaz -ide, desipramine.[10,11]

Mechanism of action of febuxostat involves the inhibition of xanthine oxidase enzyme. It binds to molybdenum pterin centre which is the active site of xanthine oxidase thereby blocking it. The enzyme xanthine oxidase is needed to oxidise hypoxanthine and xanthine to uric acid. This enzyme is inhibited by febuxostat, thereby reducing production of uric acid (Fig. 2). Therefore, helps in treatment of gout.[11,12]

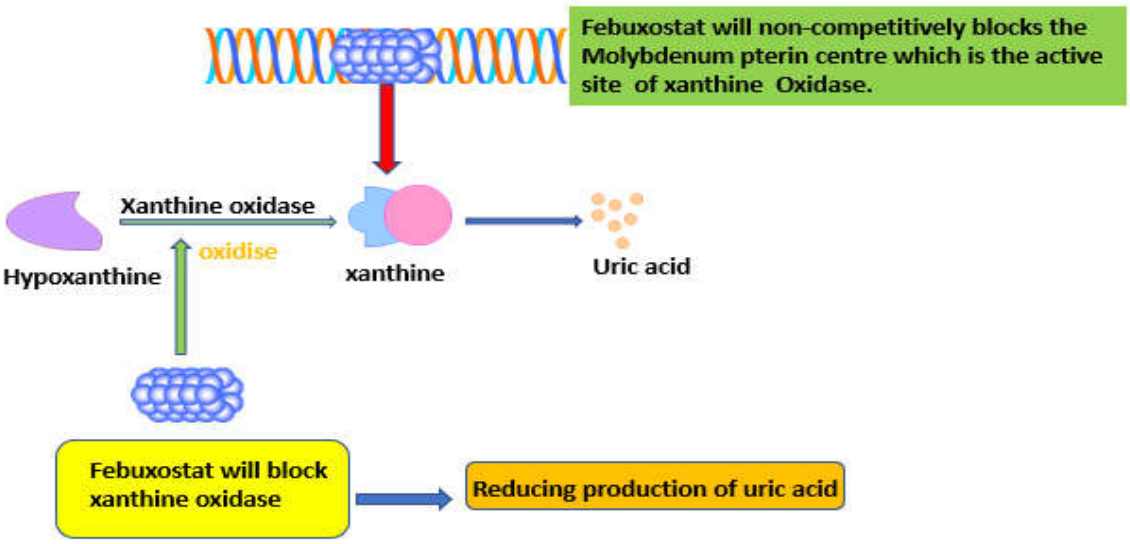

Figure.2. Mechanism of action Febuxostat.

Febuxostat is a 1,3-thiazole-carboxylic acid derivative: 4-methyl-1,3-thiazole-5-carboxylic acid which is substituted by a 3-cyano-4-(2-methylpropoxy) phenyl group at position 2. The physical properties of febuxostst[13] are presented in Table 1.

Table 1: Physical and chemical properties of Febuxostat.

Febuxostat	Uloric by Takeda Pharmaceuticals America (Trade name)
Molecular weight	316.374 g/mol
IUPAC name	2-[3-Cyano-4-(2-methylpropoxy)phenyl]-4-methyl-1,3-thiazole-5-carboxylic acid.
Molecular formula	$C_{16}H_{16}N_2O_3S$
Melting point	238-239°C

Methods of Analysis

Various methods for the quantification of febuxostat have been reported:

- UV spectrophotometric method
- High performance liquid chromatography with fluorescence detection
- Reverse phase-High performance liquid chromatography (RP-HPLC)
- High-performance thin-layer chromatography (HPTLC)
- Spectrofluorimetric method
- X-ray diffraction method
- Liquid-chromatography-tandem mass spectrometry (LC-MS)
- Ultra-performance liquid chromatographic (UPLC)
- Thin-layer chromatographic method combined with fluorescence detection

UV spectrophotometric method

Febuxostat determination by UV spectrophotometer is mostly used because of simplicity, rapidness, and cost-effectiveness. Sheth M. et al. in 2012 developed a simple and cost-effective spectrophotometric method as it prevents the use of hazardous and costly organic solvents to determine the amount of febuxostat present in a tablet dosage form. Different absorbance was measured at different wavelength between two equi-molar solutions of analyte which shows different spectral characteristics. By adjusting the pH of analyte (by aqueous solution of acid and base) the spectral properties of analyte can be adjusted. A Simadzu UV Spectrophotometer 1800 with 1.0 cm matched quartz cells was used. Two specific signals/peaks at 260nm and 315nm with +ve and -ve absorbance, respectively, were seen by putting febuxostat in N/10 sodium hydroxide in reference cell and same in N/10 hydrochloric acid in sample cell. Two maxima were calculated and then plotted against concentration to find out the amplitude. The result showed linear calibration curve at the concentration range of 5-25 µg/mL (r^2 = 0.999), with detection limit of 0.58 µg/mL. Stock febuxostat solution was prepared by dissolving 10 mg of working standard in 10 mL of methanol. Working standard solutions with concentration ranging from 5-25 µg/mL were prepared with 0.1M HCl and 0.1M NaOH, the volume was then adjusted to obtain a series of equimolar solutions of Febuxostat. Different spectra were obtained by putting acidic form (in 0.1M HCl) in reference cell and basic form (in 0.1M NaOH) in sample cell. This method can be used for general quality control test as it showed good results.[14]

Bagga P. et al. in 2011 designed a simple and cost-effective spectrophotometric method for analysis of pure febuxostat in drug formulation. Methanol was chosen as solvent system. The linearity range was 0.2-15 µg/mL at wavelength 315nm. The absorbance was found to increase linearly with increasing concentration of febuxostat. 0.1743 µg/mL & 0.5281 µg/mL were the values of limit of detection and limit of quantification. The result showed high accuracy and precision. This method could be used to determine febuxostat in drug formulations without any common excipients.[15]

Lakade S. H. et al. in 2011 developed Simple, rapid, accurate and sensitive UV- spectrophotometric method for quantification of febuxostat in pharmaceutical formulation. This method has an absorption maximum at 316nm and it obeys Beer's law at concentration value of 2- 20 µg/mL. The slope and intercept of the equation of the regression line were 0.07931 and 0.001. Correlation coefficient was found to be 0.9998. This method being simple, cost effective and less time consuming can be used for febuxostat estimation in different dosage forms.[16]

High performance liquid chromatography (HPLC) with fluorescence detection

Kamel B. et al. in 2019 developed a HPLC method coupled fluorescence detection so as to increase the sensitivity, reduction of complexity and to shorten sample preparation process time. Acetonitrile was used to precipitate protein present in plasma samples taking 2-naphthoic acid as internal standard and screening at wavelengths of 320 and 380 nm. A Luna C18 column was used with 8 min of analysis time, and mobile phase comprising of glacial acetic acid (0.032%) in acetonitrile : water (60:40, v:v) having injection volume 10 µL and a flow rate of 1.5 mL/min. Calibration curve ranged from 0.005 to 10.00 µg/mL. The results showed that the inter-day accuracy and imprecision of the quality control samples (0.0075, 0.015, 3.00 and 9.80 µg/mL) was 90–115% and ≤ 14.5%, respectively.[17]

Reverse phase-High performance liquid chromatography (RP-HPLC)

Gandla K. et al. in 2012 developed a simple, accurate, rapid, and selective method of RP-HPLC and validated it to determine febuxostat and ketorolac in bulk drug formulations. Waters C18 column (250×4.6 mm, 5µ particle article size) was used for chromatographic separation of compounds. The mobile phase was methanol and ammonium acetate buffer (adjusted to pH 6.0 with 1% orthophosphoric acid) in the ratio of 60:40, v/v. At wavelength 321nm, effluent was detected with a flow rate of 1mL/min. Retention time for ketorolac and febuxostat was 3.96 min and 2.62 min, respectively. In the concentration value of 5-30µ g/mL and 10-60 µg/mL for febuxostat and ketorolac, respectively, linearity was observed. This method could be used for routine analysis of ketorolac and febuxostat.[18]

Muvvala S.S. et al. in 2012 created an sensitive, accurate, robust and precise RP-HPLC method to measure the concentration of febuxostat in bulk formulations. By the use of Nucleosil C18 (250 x 4.6mm, 5µm) column, chromatographic separation was done. A solution of 10 mM amm. acetate buffer (pH 4.0 adjusted with 0.2% triethylamine) and acetonitrile with the ratio (15:85, v/v), was used as mobile phase having a flow rate 3.45±0.05min. Detection was performed at 275nm. The method was proposed to be linear in the range of 50.0-400.0 µg/mL. The values for detection limit and quantization were 9.98 and 30.23 µg /mL, respectively. The method has been found promising for quantitative analysis of febuxostat in bulk preparations.[19]

High-performance thin-layer chromatography (HPTLC)

El-Yazbi F.A. et al. in 2018 developed an accurate, specific and reliable HPTLC methods for analysis of febuxostat alone (method A) and febuxostat/diclofenac combination (method B) in human plasma. Method A: ethyl acetate-methanol-water (9:2:1, v/v/v) was used as mobile phase to separate febuxostat from plasma, and then it was analyzed by densitometry at λmax 315 nm. Method B was used for the determination of febuxostat and diclofenac simultaneously in human plasma. Mobile phase was comprised of a mixture of petroleum ether-chloroform-ethyl acetate-formic acid (7.5:1:2.5:0.25, v/v/v/v). By the help of densitometry at their iso-absorptive point (289 nm), febuxostat and diclofenac were quantified. Between 0.1-7 µg/mL, calibration plots of febuxostat were linear in both methods A & B. In method B, diclofenac exhibited linear response in the value of 0.08-8 µg/mL.[20]

El-Yazbi F.A. in 2016 designed an accurate, precise, economic and specific high-performance thin-layer chromatographic (HPTLC) method for simultaneous determination of febuxostat and diclofenac. Silica gel 60 GF254 plates (precoated) with chloroform–methanol 7:3 (v/v) as the mobile phase were used for chromatographic separation. At Wavelength 289nm, developed plates were scanned. For the determinations of such a mixture, derivative ratio & ratio difference spectrophotometric methods were established. The calibration plots were found linear in the range of 0.01–0.55 and 0.02–0.60 µg/band, for febuxostat and diclofenac. The calibration plots/graphs were linear between 2–14 and 4–18 µg/mL for febuxostat and diclofenac. This method being simple and specific could be used for quantification of febuxostat and diclofenac in raw materials and tablets.[21]

Hosny N.M. et al. in 2018 developed a rapid and simple thin-layer chromatography method using fluorescence detection and validated for the determination of a febuxostat-montelukast simultaneously in plasma. In this method, precoated silica gel G 60F254 plates with chloroform: methanol (9:1, v/v) as the mobile phase were used. The mobile phase was optimized and selected for development gave compact bands (RF values were 0.28 and 0.63 for febuxostat and montelukast, respectively). Using a K400 optical filter after excitation at 322 nm, emission intensities were measured. The linear regression data for calibration graphs found to be in excellent linear relationship (r^2 = 0.9990 and 0.9996 for febuxostat and montelukast, respectively), and the range of linearitywas 10.0-800.0 ng per band for both the drugs. As per ICH-directions, this method was validated for precision, robustness, accuracy and selectivity. Limits of detection and quantitation were also determined. This method was

found to be reproducible and selective for the analysis of a mixture of febuxostat and montelukast.[22]

Spectrofluorimetric method

Atia N.N. et al. in 2019 developed an super-sensitive, rapid, specific, and cost-effective spectrofluorimetric method and validated it to determine febuxostat in tablets. Enhancement of the fluorescence intensity of an acidic aqueous solution of febuxostat was obtained by using 1% w/v sodium dodecyl sulphate (SDS) as micellar system. About 2 & 30 folds improvement of the relative fluorescence intensity (RFI) of febuxostat great was observed compared to the acidic aqueous solution, and the aqueous solution of febuxostat. Relative fluorescence intensity was observed at λmax 336 nm/ λmax 410 nm. A linear relationship was observed between the fluorescence intensity of the formed febuxostat-SDS micellar system, and the concentration of febuxostat. The linearity range was found to be 0.100–25.0 ng/mL of the developed method with detection and quantitation limits of 15.08 & 45.71 pg/mL.[23]

X-ray diffraction method

Qiu J.B. et al. in 2015 developed and characterised a quantified method by using powder X-ray diffraction for different polymorphic forms of febuxostat. A continuous scan was performed at a scan rate of 3 minute. The linear regression analysis result showed fine linear relationship with r^2=0.9985 in respect to peak area in the concentration value 10–60 wt.%. Limits for detection and quantitation were 0.5% and 4.6%, respectively. This method was sensitive, repeatable and accurate and proposed for febuxostat polymorphs quantification in mixtures.[24]

Liquid-chromatography-tandem mass spectrometry (LC-MS)

Wang H. et al. in 2013 developed a sensitive and rapid analytical method consisted of liquid chromatography tandem/coupled with mass spectrometry (LC-MS). For the analysis of febuxostat in human plasma using d7-febuxostat as the internal standard, detection with positive ion electrospray ionization was performed. Capcell PAK C18 column (4.6x100 mm, 5 mm) was used for chromatographic separation. The mobile phase was acetonitrile: 5mM ammonium acetate: formic acid (85:15:0.015, v/v/v) with a flow rate of 0.6 mL/min. For quantitation, the precursor-to-production transitions m/z 317, m/z 261 (febuxsotat) and m/z 324 m/z (261+262) (d7-febuxostat) were used. To achieve multiple reactions monitoring, an Agilent 6460 electrospray coupled mass spectrometer was operated. The results were linear at the value of 10.0–5000 ng/mL, and the final analysis time for each chromatograph was found to be 3 min.[25]

Gabani B.B. et al.in 2020 found and validated a sensitive, selective and fast LC-MS/MS method for simultaneous determination colchicine and febuxostat present in rat plasma. Using 10% tertbutyl methylether in ethylacetate taking colchicine-d6 as an internal standard, febuxostat and colchicine were extracted. The mobile phase was 5 mM ammonium-formate having 0.025% formic acid in acetonitrile (20:80, v/v). Eclipse XDB-C18 column was used for chromatographic separation of the drugs with flow rate of 5.0µL and 0.9 ml/min. Febuxostat and colchicine were detected by +ve electro-spray ionization in multiple reaction monitoring using transition pair (Q1 → Q3) of m/z 400.10 → 358.10 and 317.05 → 261.00, respectively. The results showed linear assay in the value/range of 0.25-254 and 2.60-622 ng/mL for colchicine and febuxostat, respectively.[26]

Ultra-performance liquid chromatographic (UPLC)

Sahu. K. et al. in 2013 developed a faster reverse phase new ultra-performance liquid chromatographic (UPLC) method for stress testing of febuxostat. It was done in acidic, neutral, alkaline, oxidative, photolytic, and heat stresses under various ICH stress conditions. This method was proposed of giving rapider retardation/retention times, requiring lesser quantities of solvent and maintaining a good resolution. Photolytic studies were done by exposing the drug to sunlight (60,000–70,000 lux) for two days. For thermal degradation, the drug was put to 50 °C for sixty days in a heated air oven. On UPLC BEH C18 column having isocratic mode (acetonitrile: ammonium acetate buffer (pH 4.5), 70:30 v/v) at flow rate of 0.2 mL/min, chromatographic isolation was done. In the concentration range between 10 and 50 µg/mL, a linear response of drug was observed (r^2=0.999). The values for detection limit & quantification limit were 0.150 µg/mL and 1.20 µg/mL. The validated method was found to be fast, precise and simple, and could be used for routine quality-control of febuxostat.[27]

Thin-layer chromatographic method combined with fluorescence detection

Mohamed A.I. et al. in 2018 designed a new TLC (thin layer chromatographic) method coupled fluorescence detection for specific determination of febuxostat. 60 F254 silica gel plates, toluene–ethyl acetate–methanol–glacial acetic acid; (30:10:5:0.1, v/v/v/v) as mobile phase, were used for chromatographic separation. This method was used for drug determination in human plasma and urine samples, and also for quality control of different pharmaceutical dosage forms.[28]

Conclusion

Febuxostat, a xanthine oxidase inhibitor, inhibits the formation of uric acid thereby useful in treating gout (hyperuricaemia condition). It is available in the market in different forms. Several methods of analysis of febuxostat have been reported like UV, HPLC, UPLC, HPTLC, LC-MS, X-ray, spectrophotometric, and majority of these assays are simple, rapid with excellent specificity, accurate and precise. The methods could be very useful for routine analysis and quantitative determination of febuxostat in different pharmaceutical formulations.

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Review Article

Journal of Pharmaceutical and Medicinal Chemistry
Volume 7 Number 1, January – June 2021
DOI: http://dx.doi.org/10.21088/jpmc.2395.6615.7121.3

A Review on Inductively Coupled Plasma: Mass Spectrometry with Laser Ablation

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How to cite this article:
Sai Datri A, Nataraj KS, Lakshmana Rao A, A Review on Inductively Coupled Plasma: Mass Spectrometry with Laser Ablation. J Pharmaceut Med Chem. 2021;7(1):23-32.

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Abstract

Inductively coupled plasma mass spectrometry (ICP-MS) is a kind of mass spectroscopy which is used in many diverse research fields such as earth, environmental, life and forensic sciences and in food, material, chemical, semiconductor and nuclear industries. In this sort of MS, Laser ablation (LA) ICP-MS is widely used to determine elements directly in virtually all types of solid samples with minimal sample preparation. UV lasers are widely used with ICP-MS because of their highly controllable spatial resolution (spot size) and relatively low cost. This technique is used to determine low-concentrations and even ultra-low-concentrations of elements. Atomic elements are lead through a plasma source where they become ionized. The high ion density and the high temperature in plasma provide an ideal atomizer and element ionizer for all types of samples and matrices introduced by a variety of specialized devices. Then, these ions are sorted on account of their mass. ICP-MS holds a distinctive position by virtue of its speed, sensitivity, dynamic range and elemental coverage. It can be considered as a viable alternative to ICP-Optical Emission Spectroscopy (OES) (also known as Atomic Emission Spectroscopy or AES) for fast measurement of higher concentration elements. At the same time, ICP-MS in many cases exceeds the detection capability of Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) for the determination of trace and ultra-trace elements (ng/L or ppt concentrations). One of the fastest growing areas of ICP-MS is in speciation measurement: the combination of chromatographic techniques with ICP-MS as a detector to determine the chemical form of elements in the sample. This review provides an overview of recent developments and abilities of inductively coupled plasma mass spectrometry (ICPMS) coupled with different separation techniques for applications in the field of analysis and also highlighted numerous technical improvements, over the past few years which helped to promote the evolution of ICP-MS to one of the most versatile tools for elemental quantification as Laser ablation (LA) ICP-MS. In particular, the benefits and possibilities of using state-of-the-art hyphenated ICP-MS approaches for quantitative analysis applications.

Keywords: Inductively couple plasma mass spectrometry; ICP-MS; Laser ablation; Hyphenated techniques; Quantification and Trace elements.

Introduction

Inductively coupled plasma mass spectrometry[1] (ICP-MS) is a type of mass spectroscopy which is used in many diverse research fields. This technique is used to determine low-concentrations and even ultra-low-concentrations of elements. While many sampling methods have been investigated for use with ICP-MS, some have become outdated, or remain of academic interest, such as spark ablation and slurry nebulization for solids analysis, and electro thermal vaporization (ETV) as a sample introduction device.

The most commonly used sample introduction devices in practical analytical applications include:

a. Integrated sampling systems
   - Constant flow nebulization
   - Autodilution
   - Discrete sampling
   - Hydride generation
   - On-line matrix removal
   - Low-pressure chromatography

b. Laser ablation (LA)

c. Desolvation systems

d. Chromatography techniques

Laser ablation (LA) ICP-MS is commonly used to determine elements directly in nearly all types of solid samples with nominal sample preparation. It is a highly sensitive technique with a wide analytical dynamic range from the single figure part per billion (ppb) to the percent (%) level in the solid. UV lasers are widely used with ICP-MS because of their highly controllable spatial resolution (spot size) and fairly low cost. Systems with different wavelengths and beam profiles are available depending on the types of samples to be analyzed.

This technique is greatly recognized for:

- Direct analysis of solids and powders
- It's suitable for all kinds of solid materials including geological sample types, ceramics, metals and alloys, biological and forensic samples.

Main Applications include:

- Surface mapping studies of rocks, minerals and glasses to establish elemental distribution and migration.
- Bulk sampling of metals, alloys, nonconductive polymers and ceramics for elemental content.
- Feature analysis of micro-inclusions and small spots.

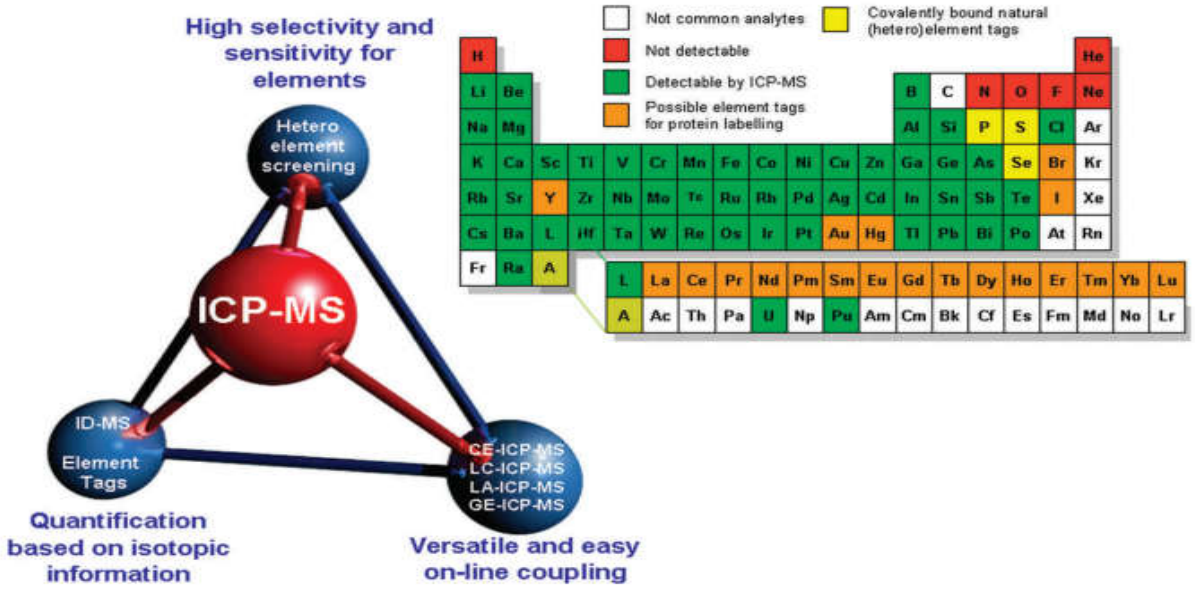

Fig. 1: Specific Features of ICP-MS[2]

History

Dr. Alan Gray of Applied Research Laboratories in Luton, UK, performed much of the early research work that led to the commercial development of ICP-MS instrumentation. Initially he worked with capillary direct current (DC) arc plasma coupled to a quadrupole mass spectrometer, he published early results and the first mass spectra acquired from plasma, in a paper in 1975[3]. This work inspired research into the use of inductively coupled radio frequency (RF) plasmas (ICP's), with some of the key developments taking place in the lab of Velmer Fassel at Iowa State University in collaboration with Dr. Gray in 1978. That leads to the development of first commercial instruments in the early 1980's based on the publication by Houk *et al.*[4] which demonstrated the possibilities offered by the ICP-MS technique. These systems were derived from parts of two existing technologies – the argon ICP, already in use in ICP-OES, and the quadrupole mass spectrometer, then being applied in fields such as Gas Chromatography Mass Spectrometry (GC-MS) and residual gas analysis. Some changes were necessary to allow the ICP to operate in physical contact with the grounded spectrometer interface, but the characteristics of these existing technologies were well matched and the performance of the first systems was impressive. Although the early ICP-MS systems were expensive, large, complex, had limited automation and tended to suffer from significant signal drift, the obvious benefits of a multi-element technique with low limits of detection and a simple mass spectrometric data output (including isotope ratio information) led to acceptance of the fledgling technique, particularly among those involved in research and geological applications. Application of the technique in laboratories where reliability, stability and automation were a high priority, led to rapid improvement of the commercial instruments and ultimately to the small, reliable, stable and highly automated systems available today. ICP-MS systems with magnetic sector and time-offlight mass analyzers have also been commercialized, but the quadrupole-based systems remain the

configuration of choice by a very wide margin. Since the first commercial ICP-MS systems were launched, major developments have occurred in sample introduction, plasma efficiency, ion transmission, interference removal and dynamic range. Even so, the major components of a modern ICP-MS instrument can be traced directly back to the earliest systems, illustrating how brilliant the original concept was.

Principle[5,6]

The general schematic representation of processes in ICP-MS from sample introduction to mass analysis is shown in Fig. 2. Distinct techniques used for the sample introduction based on physical state of the sample like for Nebulizers for liquids and Laser for Solids. Among all the Laser ablation is one technique for sample introduction for solids. For all most all laser ablation systems used with ICP-MS utilize a neodymium doped yttrium aluminum garnet crystal laser to generate a high intensity pulsed light beam at a fundamental frequency of 1064 nm. This is frequency quadrupled or quintupled to the analytically useful ultraviolet wavelengths of 266 nm or 213 nm respectively. The beam is focused onto the sample surface in an ablation chamber or cell, which is purged with argon or helium. The beam diameter can be precisely set by software-controlled apertures to generate variable "spot" sizes typically from <5 µm to 750 µm based on the application and laser being used for the analysis. The laser light couples to the surface of the sample, creating a very rapid heat, which leads to volatilization or ablation of the matrix. The laser induced aerosol which is created is then move to the ICP in an argon carrier gas stream where it get decomposed, atomized and ionized, before sending into the mass spectrometer vacuum system for analysis.

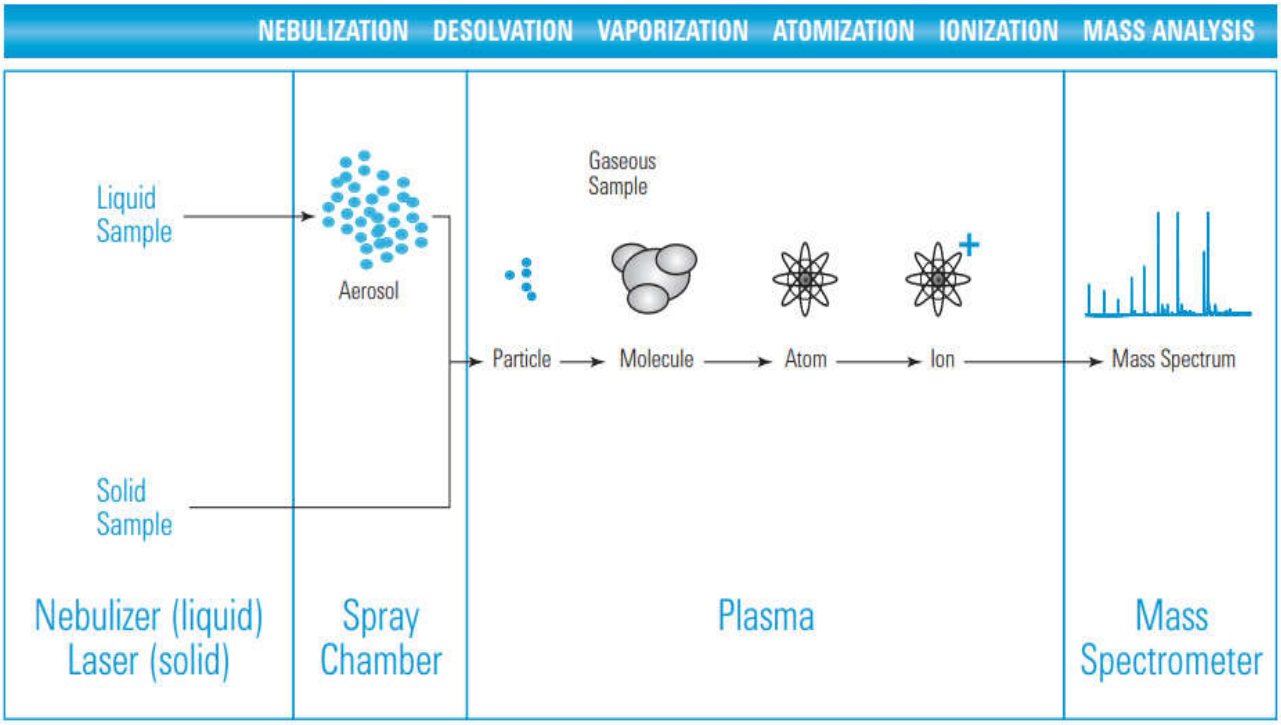

Fig. 2: Schematic representation of processes in ICP-MS from sample introduction to mass analysis.

Theory[7]

Laser ablation (LA) ICP-MS is extensively used to quantify elements directly in almost all types of solid samples with least sample preparation. It is a extremely sensitive technique with a broad analytical dynamic range from the single figure part per billion (ppb) to the percent (%) level in the solid.

The sample solution is introduced through a peristaltic pump into the device, where it becomes nebulized in a spray chamber. From there, it enters into an argon-plasma, which has a temperature of 6000-8000 K. Inside the plasma torch, solution is removed from the sample and also atomization and ionization occur. Only a small amount of the ions produced into the plasma and further infiltrate to the mass-spectrometer part.

The mass-spectrometer part consists of: An interface, in which a small amount of the free ions generated by the plasma are transmitted. During this process, the ions transfer from an environment with extremely high temperature and atmospheric pressure to a compartment at room temperature and high vacuum (< 0,001 Pa). After which the process continued with electrostatic lenses, which focus (positive) ions onto the entry to the true mass-spectrometer. The true mass-spectrometer in the GI device has a quadrupole, composed of 4 metal rods which separate the ions on basis of their mass by a kind of resonance principle. Then it reaches, electro-multiplier (a special type of detector) containing active surfaces, which enhances the signal from one colliding ion so that a measurable pulse is generated and finally, the electronics counts and sorts the pulses and relates them to the corresponding mass. This selection can be accomplished in milliseconds, so that a complete spectrum can be acquired within one second.

Instrumentation[8-15]

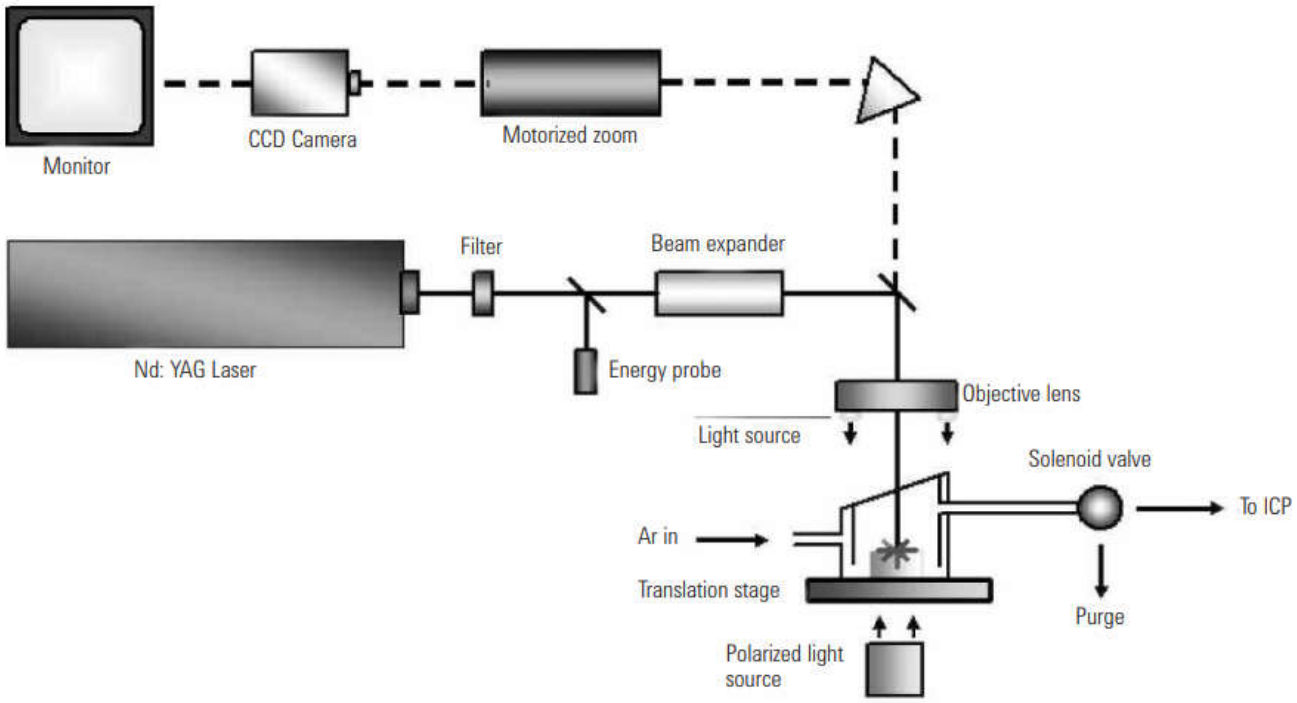

Fig. 3: Instrumentation of neodymium doped yttrium aluminum garnet crystal Laser Ablation system.

Parts

- Sample Introduction
- Argon Plasma
- ICP-MS Interface
- Vacuum System
- Ion Focusing
- Collision/Reaction Cells
- Mass Analyzer
  - Quadrupole
  - Magnetic Sector
  - Time-of-flight (TOF)
- Detector

ICP technology was built upon the same principles used in atomic emission spectrometry. Samples are decomposed to neutral elements in high temperature argon plasma and analyzed based on their mass to charge ratios.

Sample Introduction:

The sample introduction system is one of the most important components of the entire ICP-MS system and the first step in analysis. A well-designed sample introduction system will reduce routine maintenance and enhance analytical performance. This has been achieved in ICP-MS through a distinct means. ICP-MS spectrometers can accept solid as well as liquid samples. Solid samples are introduced into the ICP by way of a laser ablation system, which is shown in Fig. 4. In this method, a laser is focused on the sample and creates a plume of ablated material which can be swept into the plasma.

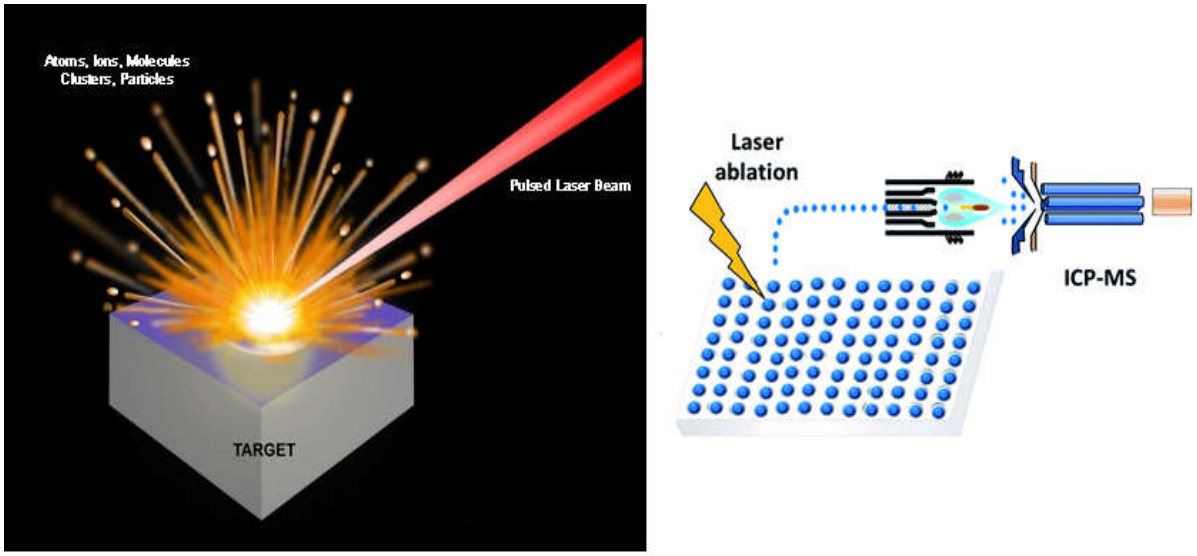

Fig. 4: Laser Ablation in ICP-MS.

Argon Plasma/Sample Ionization:

In the simplest terms, the purpose of the plasma is to form positively charged ions from the sample aerosol. To ensure good results from samples with high or varying matrices, plasma loading should be optimized to maintain high ionization temperatures while retaining good sensitivity. The goal is to achieve as high a degree of matrix decomposition and analyte ionization as possible. The sample aerosol is passed into the plasma, which is generated in a stream of argon (Ar) contained in a coupling coil and that is used to transmit radio frequency to the heated argon gas, producing an argon plasma "flame" located at the torch. The hot plasma removes any remaining solvent and causes sample atomization followed by ionization. In addition to being ionized, sample atoms are excited in the hot plasma, a phenomenon which is used in ICP-atomic emission spectroscopy. Shown to the right is an ICP torch. The aerosol moves into the bottom of the torch body. The green ports on the right side of the body are where more argon is introduced to the flow. At the top are two high quality quartz tubes and an inner alumina injector tube shown in Fig. 5.

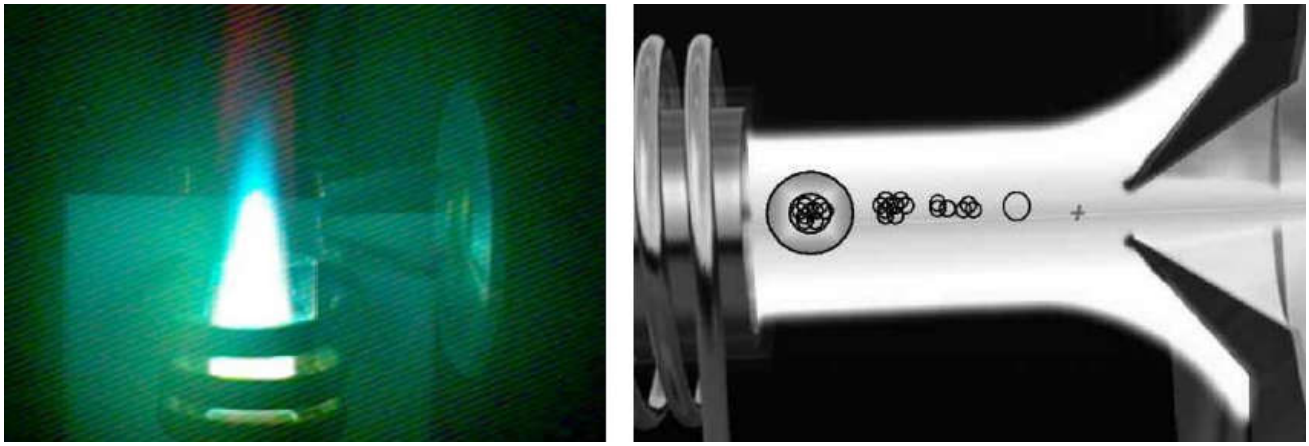

Fig. 5: Argon Plasma in Operation.

The basic steps of ion production in an ICP are:

- Sample droplets move toward the plasma and are dried
- The dried sample particles are decomposed by the plasma to form atoms (atomization)
- At this point (atomization stage), the process is optimized for ICP-OES. In ICP-OES, emitted light is determined from excited atoms as these lines are typically more stable.
- ICP-MS measurement requires an extra step: the atoms must be ionized, since the mass analyzer can only separate ions.

Thus the ICP in ICP-MS is an ion source - this requires more energy. The formation of ions from the sample atoms is achieved by the removal of a single electron. This occurs with varying ease and efficiency for different elements. This variation is usually quoted as the "Ionization Efficiency" for each element, which is a function of the first ionization potential of the element, together with estimated values for plasma electron temperature and density.

ICP-MS Interface:

The positively charged ions that are produced in the plasma are extracted into the vacuum system, via a pair of interface "cones". The cones are essentially metal plates with central orifices through which the ions pass which is shown in Fig. 6. Generally, atomization/ionization occurs at atmospheric pressure, the interface between the ICP and MS components becomes crucial in creating a vacuum environment for the MS system. Ions flow through a small orifice, approximately 1 millimeter in diameter, into a pumped vacuum system. Here a supersonic jet forms and the sample ions are passed into the MS system at high speeds, expanding in the vacuum system. The entire mass spectrometer must be kept in a vacuum so that the ions are free to move without collisions with air molecules. Since the ICP is maintained at atmospheric pressure, a pumping system is needed to continuously pull a vacuum inside the spectrometer. In order to most efficiently reduce the pressure several pumps are typically used to gradually reduce pressure to 10-5 mbar before the ion stream reaches the quadruple. If only one pump were used, its size would be excessive to reduce the pressure immediately upon entering the mass spectrometer.

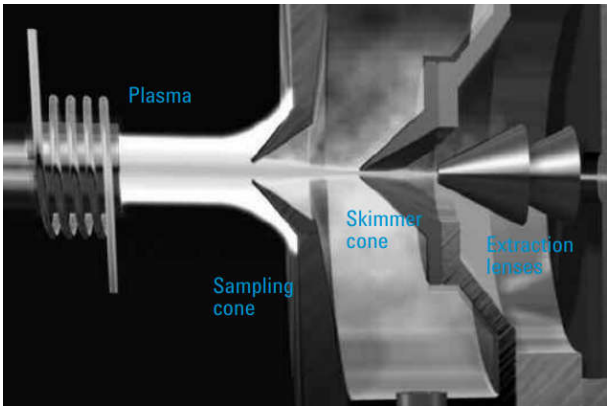

Fig. 6: ICP-MS Interface.

Vacuum System:

Mass spectrometers work most efficiently at low pressure (high vacuum). The maintenance of a high vacuum in the

analyzer region is essential, in order to reduce the background and scattering effects that a high level of residual gas molecules would cause. The preferred configuration in both early and current commercial instruments is for a 3 stage differentially pumped vacuum system comprising the interface, intermediate and analyzer stages at progressively lower pressures. The typical vacuum configuration for commercial ICP-MS instruments is for the interface stage to be evacuated using a rotary vane pump, which is switched off when the ICP-MS is in "standby" mode, to allow access to the interface cones and ion lenses for maintenance. The intermediate and analyzer vacuum stages are typically pumped by two separate turbo-molecular pumps or by a single, dual-stage pump. A "backing" rotary pump removes exhaust from the turbo-molecular system. The intermediate and analyzer vacuum stages are typically isolated from the interface region by a gate valve, which seals the high vacuum region when the interface pump is switched off.

This allows routine maintenance without requiring the high vacuum pumps to be switched off, so the vacuum is maintained and start-up times are minimized. The gate valve is under pneumatic or solenoid switch control, such that any power, coolant or gas failure or plasma shutdown causes the valve to shut automatically, avoiding sudden loss of vacuum.

Ion Focusing:

Electrostatic plates, known as ion "lenses", keep the ions focused in a compact "ion beam" as they pass through the vacuum system to the final chamber, where the mass spectrometer (MS) and detector are housed which is shown in Fig. 7. The ion lenses perform a second essential function of separating the ions from the photons and residual neutral material, which is achieved by using the electrostatic fields in the lenses to deflect the ions thus separating them from the photons and neutrals. It has a high transmission off-axis or Omega lens arrangement that separates the positively charged ions from the photons and neutral particles, which would otherwise reach the detector and increase random background noise.

In order to prevent the loss of ions from the beam, the ion lenses are used to focus and transfer charged species efficiently to the mass spectrometer entrance aperture. While several different ion lens designs have been used in ICP-MS, the typical arrangement is to use one or more cylindrical lenses, to which a voltage can be applied. When the positive ions generated by the plasma pass through the electrostatic field in the lens system, they are attracted to negative and repelled from positive fields, so can be manipulated in the required trajectory.

The ion lens system may consist of a simple, single cylindrical electrostatic lens, which has the virtue of low cost and simple operation, but has limited flexibility. Alternatively, a multi-component ion lens design may be used, which increases cost but allows greater flexibility of optimization. Early designs of lens systems utilized a grounded metal disc, known as a "photon" or "shadow" stop, on the axis of the instrument, to block the direct line from plasma to detector.

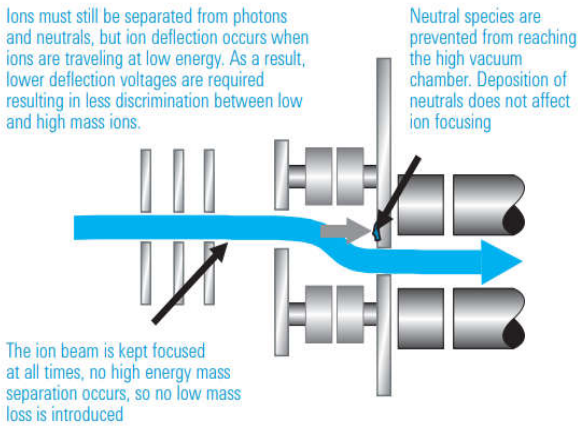

Fig. 7: Schematic of off-axis ion lens design.

Collision/Reaction Cell:

Collision/Reaction cells (CRC's) are helps to remove spectral. There are various configurations of CRC but fundamentally the device consists of an ion guide, which is enclosed in a cell that can be pressurized with a gas and is positioned after the main ion lenses. The gas interacts with the ion beam to remove polyatomic interferences in any of these two ways like Reaction Mode, where the gas reacts with an interference to convert it to a different species or Collision Mode, where the gas collides with the polyatomic interference, causing it to lose energy. Since polyatomic species are large, they undergo more collisions than do analytes, and so lose more energy. The lower energy interference is then separated from the higher energy analyte by energy discrimination (ED).

Mass Analyzer:

Finally, Ions pass from the ion lens system into the analyzer vacuum stage, where they are separated by the quadrupole, according to their mass to charge ratio. The most widely used mass analyzer in ICP-MS is the quadrupole because of its ease of use, robustness, mass range, high scanning speed and relatively low cost. The other analyzers that have been used in ICP-MS are magnetic sector or double focusing and time of flight (TOF).

Quadrupole: It is a sequential mass filter, which separates ions based on their mass to charge ratio (m/z). It consists of two pairs of parallel cylindrical rods, arranged in a square, on the axis of the ion beam which is shown in Fig. 8. A varying or AC voltage, operating at high frequency, plus a DC voltage is applied to the two pairs of rods. The AC (same voltage but out of phase between the 2 pairs of rods) and DC (positive on one pair and negative on the other) voltages give a dynamic hyperbolic electric field, in which any ion above or below the set mass of the quadrupole enters an unstable trajectory and is lost from the ion beam.

Combining the AC and DC components produces a narrow band pass filter that allows only a narrow range of masses to be transmitted. By varying the AC and DC fields, but keeping the ratio between them constant, different masses can be selectively allowed to pass through the filter. Since these voltages can be adjusted very rapidly, the elemental mass range from 2 to 260amu

can be scanned very quickly, giving a mass spectrum for all elements and their isotopes (Li to U), which is acquired virtually simultaneously.

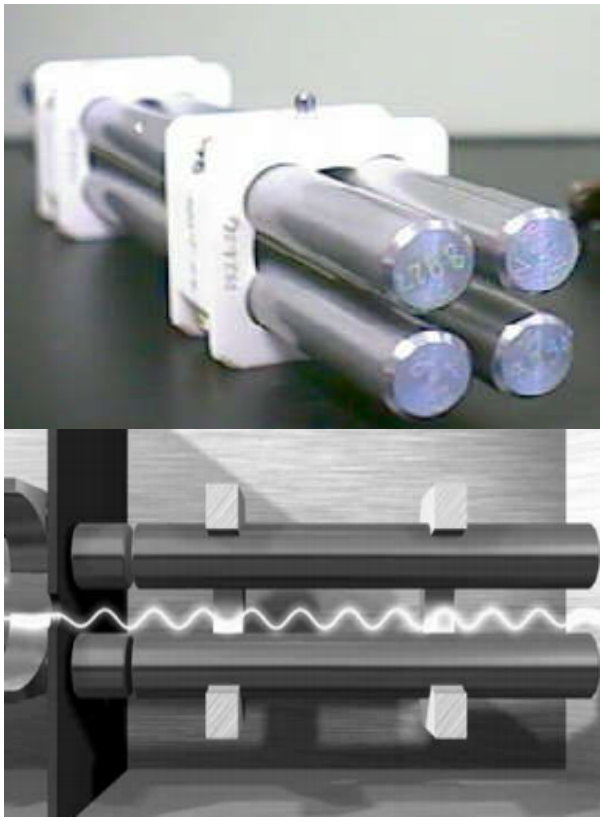

Fig. 8: Schematic of Quadrupole Analyzer.

Magnetic Sector or Sector Field Analyzers: These types of analyzers are typically employed where mass resolution significantly higher than unit resolution is required. The systematic of this type of analyzer is shown in Fig. 9.

The principles can be explained as follows:

- Ions sampled from the plasma are first accelerated in the ion optic region before being focused into the variable entrance slits – this stage determines resolution.
- The ions then enter an electromagnet induced magnetic field which deflects different masses through different angles.
- The next step is often referred to as "energy filtering": - Ions enter an electrostatic sector, where they are filtered or resolved according to their kinetic energy (energy resolution).
- Ion detection done by using multiple Faraday cups are used in multi collector systems. Multi collector systems are optimized for high-precision isotope ratio analysis of a large range of elements. They operate at a maximum resolution of around 3500. These systems are not well suited to making trace concentration measurements because of poor signal to noise on the Faraday cup detectors.

Fig. 9: Schematic of Magnetic Sector Analyzer.

Time of Flight Mass Analyzer: In a time of flight mass analyzer which is shown in Fig. 10, a uniform electrostatic pulse is applied to all ions at the same time, causing them to accelerate down a flight tube. Lighter ions get higher velocities and reach the detector first, so the mass-to-charge ratios of the ions are determined by their arrival times. These analyzers have also been used in ICP-MS for applications where many masses are determined in short lived transient signals e.g. laser ablation studies. This is because the time of flight mass spectrometer separates the ions and delivers all masses to the detector with a very short time delay, allowing many thousands of full mass scans to be acquired per second, giving a virtually simultaneous measurement.

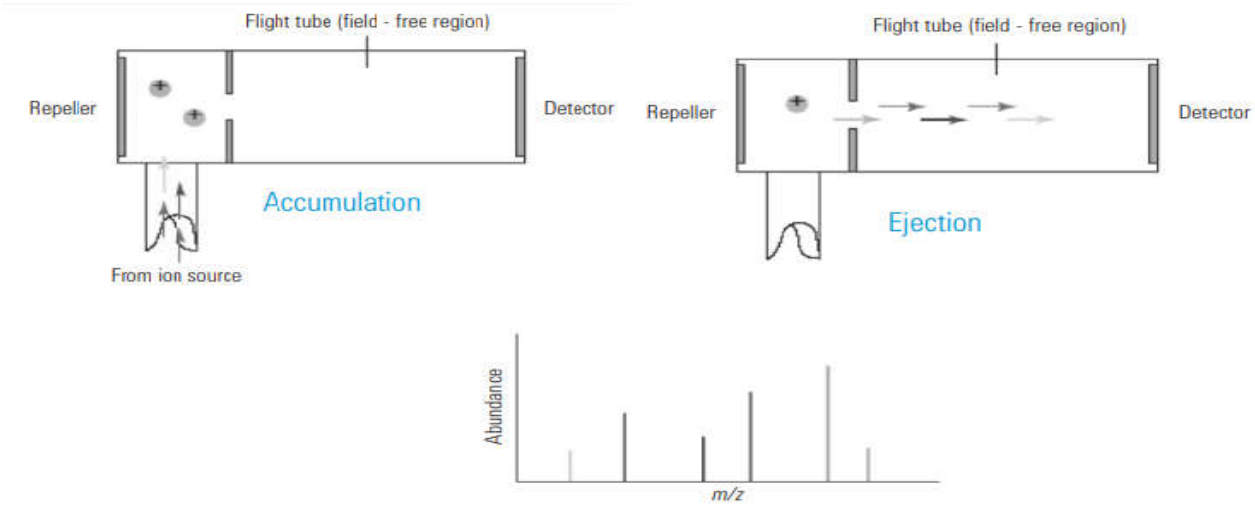

Fig. 10: Schematic of Time of Flight Mass Analyzer.

Detector:

The detector is responsible for the distinctiveness of very high sensitivity and low random background, for which the technique is famous. The reason for the high sensitivity is that the detector used in virtually all modern ICP-MS instruments is a so-called "electron multiplier" device, which means that it can generate a measurable signal pulse from the impact of a single ion which is shown in Fig. 11. To make best use of this sensitivity, it is essential that the arrival of an ion can be reliably distinguished from any random background noise arising either from the vacuum and spectrometer system or from the electronics.

The basic principle involved in these detectors is at the first, a positive ion arrives at the mouth of the detector, and it is deflected onto the first dynode, which is held at a high negative voltage. The impact of the ion releases several free electrons from the dynode surface, which are repelled from the high negative voltage at the front and strike the next dynode. Each electron which strikes the second dynode releases several electrons from that surface and so on down the many stages of the detector – hence the name "electron multiplier". By the time the electron cascade reaches the final dynode, the multiplication factor has built up a pulse large enough to be measured reliably as an ion "count".

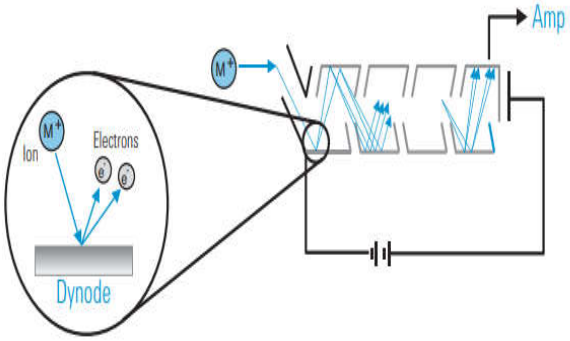

Fig. 11: Schematic of Electronic Multiplier.

Detection Limits[16]

One of the great advantages to ICP-MS is extremely low detection limits for a wide diversity of elements. Some elements can be measured down to part per quadrillion ranges while most can be detected at part per trillion levels. The Table 1 shows some common detection limits by element.

Table 1: Detection Limit of Some Elements.

Element	Detection Limit (PPT)
U, Cs, Bi	less than 10
Ag, Be, Cd, Rb, Sn, Sb, Au	10-50
Ba, Pb, Se, Sr, Co, W, Mo, Mg	50-100
Cr, Cu, Mn	100-200
Zn, As, Ti	400-500
Li, P	1-3 ppb
Ca	less than 20 ppb

Advantages[17]

- Wide elemental coverage – nearly all elements can be measured by ICP-MS, including alkali and alkaline earth elements, transition and other metals, metalloids, rare earth elements, most of the halogens and some of the non-metals.
- Performance - high sensitivity and low background signals combine to give very low detection limits (sub-ng/L – parts-per-trillion (ppt) in most cases)
- Rapid analysis times – with a high speed scanning quadrupole analyzer, measurement of a full suite of elements takes only about 4 minutes per sample
- Extensive analytical operational range – up to 9 orders in a single acquisition
- Isotopic information
- Excellent chromatographic detector

Applications of Laser Ablation (LA) ICP-MS[17-21]

- *Environmental*
  - The identification of trace elements in "clean" samples such as drinking water, rain water and air samples.
  - The quantification of elements over a wide concentration range in wastewater, sewage sludge, trade effluents, landfill leachates, soil and sediment digests and biota.
  - The identification of trace and ultratrace elements in high matrix samples such as open ocean seawater.
- *Food and Agriculture*
  - Trace element monitoring at almost every stage of food production.
  - Measuring isotope ratios in elements provided as trace element supplements.
  - Quantifying metals in proteins to monitor elemental absorption from the diet.
  - Semiconductor
  - Analysis of ultra-pure water (UPW) and process chemicals used in semiconductor manufacture.
  - Quality control of semiconductor devices.
- *Clinical and Pharmaceutical*
  - The identifying of trace elements in urine, blood and serum.
  - Quantification of heavy metals and/or metal species in drug product.
- *Geological*
  - Categorization of rocks and minerals.
  - Broadcast samples in mining exploration, product quality and ore processing.
  - Isotope ratio determination for geochronology.
- *Nuclear*
  - Nuclear fuel production: impurity analysis of fuels intermediate compounds and fuel cladding materials.
  - Nuclear power plants: monitoring of primary cooling water for corrosion and monitoring of moderator (boron) isotope ratio.
  - Effluent discharge monitoring.
  - Monitoring of workforce – clinical sampling and workplace monitoring.

- ***Forensic***
  - Accurate measurement of elemental "fingerprint" in crime scene evidence to characterize and identify materials.
  - Discriminating elemental and isotopic differences of solid samples directly at the part per billion level using laser ablation LA-ICP-MS.
- ***Chemical, Petrochemical***
  - Analysis of trace metal concentrations in petrochemical samples.
  - Trace element levels in printer ink.
  - Speciation measurement of petrochemical samples.

Improvements Required For ICP-MS

When compared to all the other available methods of elemental analysis, ICP-MS comes closer to being the ideal method, but still need some improvements in.

- Speciation approaches must be devised or refined.
- Matrix interferences must be better understood and controlled.
- Less expensive instruments for the elimination of spectral interferences must be developed.
- Alternative sources and mass spectrometers should be considered and evaluated.
- Sample-introduction efficiency must be improved.
- Sample-utilization efficiency ought to be raised.
- Precision must be increased.
- Instrumentation should be reduced in price and made simpler to use.

Conclusion

The outstanding instrumental developments for the period of the last decade in the field of elemental mass spectrometry and its application as a (hetero)element specific detector, as well as the continuous progress in the development of new hyphenated techniques, has resulted in a growing interest in such technologies and their unique analytical properties. As indicated by the broad range of examples ranging from environmental analysis of emerging compounds to proteomics related topics, such as protein phosphorylation studies or absolute protein quantification, ICP-MS has the potential to become a key technology for quantification, especially because of its unique characteristics, such as compound-independent ionization behavior, sensitivity, and robustness, which represent the main strengths of ICP-MS. With respect to most of these criteria, determination of trace elements by ICP MS is performing enormously and is uncontested by other MS techniques. On the other hand, it is a unique and outstanding advantage of ICP MS to use inexpensive, unspecific, certified element standards, allowing a quantitative control on elemental losses, species decomposition or contamination in each single step of an experiment. Whereas molecular fragmentation is used to determine the structure of unknown compounds, in plasma, atomized elements provide convenient and efficient access to high sensitivity for target-element orientated quantitation and the discovery of relevant unknown compounds and in the same process quantifying their relative mass contribution to the total content. Among all, Laser ablation (LA) ICP-MS is commonly used to determine elements directly in nearly all types of solid samples with nominal sample preparation. It is a highly sensitive technique with a wide analytical dynamic range from the single figure part per billion (ppb) to the percent (%) level in the solid. UV lasers are widely used with ICP-MS because of their highly controllable spatial resolution (spot size) and fairly low cost. It is probable that in the future, offsprings of today's instruments will contain both types of ion sources, allowing researchers to work with only one combined analytical system and still satisfying the research needs.

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