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Determination of Bromide from Bacopa Monnieri (Brahmi) and Other Herbal Plants by Ion Chromatography

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Abstract: ***An Ion Chromatography method using suppressed conductivity detection for the quantitation of Bromide from Bacopa Monnieri (Brahmi) and other Herbal plants was developed and validated. Separation of Bromide from other anions was done using high capacity anion exchange column with a flow rate of 1.0ml/min. The calibration curve of standard Bromide showed good linearity of more than 0.999 of the correlation coefficient. The Limit of Detection and Quantification was 0.008mg/L & 0.028mg/L respectively. The relative standard deviations of intra and inter day analysis of Bromide was less than 3.0%. The proposed method was successfully applied to determine Bromide in various herbal plant extracts which are available at local market in India.***

Keywords: Herbal, Bromide Ion Chromatography, Suppressor, Conductivity, Bacopa Monnieri, Brahmi, Tulsi, Ginger, Amla, Ayurveda

1. Introduction

Bacopa Monnieri, which is commonly known as Brahmi in India, has many medicinal uses especially in herbal and ayurvedic field. It reduces stress and anxiety. It also helps to ease Alzheimer's disease. It is full of antioxidants and strengthens immune system, which is essential for living a healthy life [1]. Similarly, other herbal and ayurvedic medicines are also meant to improve quality of human life.

Methyl Bromide is utilized as a soil fumigant fundamentally in blends with chloropicrin and the C3 chlorinated hydrocarbons (dichloropropane and dichloropropene) to control nematodes, bugs, and weeds. In blends with chloropicrin it has been utilized widely since 1967 as a pre-plant soil fumigant to control soil-borne sicknesses of strawberries, especially Verticillium wither. A great part of the methyl bromide diffuses all through the surface 2 to 8 feet of soil, its vast majority in the long run disseminating into the environment. The sum that remaining parts in the soil is either hydrolyzed or decayed by microorganisms, in either case discharging the bromine as inorganic bromide. Right now it is a contrarily charged particle and accordingly move as water moves in the soil along the lines of chloride and nitrate. Plant species retain bromide from the soil. Sometimes, there might be phytotoxicity and at specific levels bromide might be perilous to human wellbeing. This is particularly valid if the plants or palatable bits of such plants as spinach, Brussels sprouts, strawberries, and grapes are devoured by people [2] Herbal and medicinal plants had been cultivated in same way as other vegetable and fruit plants and can uptake Bromide. In 1970, the Food and Agriculture Organization/World Health Organization recommended a bromide resistance point of confinement of 20 to 30 ppm (parts per million) for fresh fruits and 250 ppm for dried natural products [3].

In the atmosphere, Methyl Bromide exhausts the ozone layer and permits UV radiation to arrive at the earth's surface. Methyl Bromide is a Class-I ozone-draining substances (ODS) [4]. Therefore, it had been banned in most parts of world including India. Bromide was once utilized as an anticonvulsant and narcotic at portions as high as 6 g/day. Clinical side effects of bromide inebriation have been accounted for from its restorative employments. Enormous dosages of bromide cause sickness and heaving, stomach torment, trance state and loss of motion. Portions of bromide giving plasma levels of 12 mmol/l (96 mg/l plasma) produce bromism (the interminable condition of bromide inebriation), and plasma levels more noteworthy than 40 mmol/l (320 mg/l plasma) are here and there lethal (EMEA, 1997). The signs and side effects of bromism identify with the sensory system, skin, glandular emissions and gastrointestinal tract (van Leeuwen and Sangster, 1988) [4].

Ion exchange chromatography is a liquid chromatographic technique, in which ionic and strongly polar species can be well separated and detected. It can be used to detect the presence Bromide from Herbal plants using high capacity column to separate it out from other anions with suppressed conductivity detection. This method provides excellent chromatography when compared to other conventional methods and allows detection of them at low mg/l(ppm) level. The developed methodology has been validated and it is highly effective to estimate Bromide from Bacopa Monnieri (Brahmi) and other Herbal medicinal plants.

2. Experimental

2.1 Reagents and Chemicals

All chemicals used for preparation of reagents, standards and mobile phase were of analytical grade. Ultrapure deionized water (18.2 MΩ cm, Milli-Q system) was used for the preparation of mobile phase, standards and samples. Sodium Bromide (AR grade, Merck) was used for the preparation of Bromide standard, Sodium Carbonate (AR grade, Merck) and Sodium Bicarbonate (AR grade, Merck) were used to prepare eluent mixture of 4.5mM Sodium Carbonate and 0.8mM Sodium Bicarbonate. Methanol (AR grade, Merck)

was used as the extraction solvent. Samples includes Bacopa Monnieri (Brahmi) powder (Sample Identification Number (S. I. No. BM01, BM02, BM03), Tulsi powder (S. I. No. TU01), Centella Asiatica powder (S. I. No. CA01), Salvia Officinalis powder (S. I. No. SO01), Mucuna Pruriens powder (S. I. No. MP01), Ashwagandha Powder (S. I. No. AW01), Beetroot powder (S. I. No. BT01), Emblica Officinalis (Amla) powder (S. I. No. EO01), Pterocarpus Marsupium powder (S. I. No. PM01), Coleus Forskohii powder (S. I. No. CF01), Horse chestnut powder (S. I. No. HC01), Pomegranate powder (S. I. No. PG01), Licorice root powder (S. I. No. LR01), Boswellia Serrata (S. I. No. BS01), Garcinia Cambogi powder (S. I. No. GC01), Grape seed powder (S. I. No. GS01), Rosemary powder (S. I. No. RM01), Fenugreek seeds powder (S. I. No. FS01), Ginger powder (S. I. No. GG01), Terminalia Bellerica powder (S. I. No. TB01), Phaseolus Vulgaris powder (S. I. No. PV01). All samples were purchased from local Ayurvedic and Herbal stores.

2.2 Apparatus

Laboratory Extraction assembly was used for extraction of plant material consisting of 1 L Round bottom flask (RBF), Heating mantle, thimble (Extraction Chamber) and Condenser. The equipment used was Thermo Fisher Dionex Ion Chromatography Aquion system with Conductivity detector having autosampler with a 25µL loop, IonPac AS23 column (4 x 250mm) and IonPac AG23 guard (4 x 50mm) was used as separator column. The experiment was conducted using a eluent mixture of 4.5mM Sodium Carbonate and 0.8mM Sodium Bicarbonate at a flow rate of 1.0ml/min Column outlet was connected to CDRS600, 4mm Suppressor. It was then connected to Conductivity detector. Suppressor was used in recycle mode and Constant Voltage mode was applied to suppressor to decrease background eluent conductivity. Software used for data acquisition was Thermo Fisher Dionex Chromeleon (version: 7.2.9). Chromatograms were monitored simultaneously during analysis.

2.3 Procedure

Extraction of plant material

The plant material was dried in oven at temperature not more than 50° C to remove the moisture. Then, material was powdered (Coarse form) in a grinder and stored in cool and dry place.

Methanolic extraction of the plant material was carried out in Laboratory Extraction Assembly. The thimble was packed with cotton plug and filter paper at the bottom and on top of that the 100 gm of the plant powder was filled. The top of the powder bed covered with round piece of normal filter paper to ensure complete circulation of the solvent through the powder bed. About 300 ml of solvent (Methanol) was filled in round bottom flask. The RBF was then placed on Heating mantle. Extraction thimble along with condenser connected to RBF. Whole assembly was connected and balanced by using stand and clamps. Water circulation through condenser was started. Remaining portion of 200 ml of Methanol was added on top of the powder bed through condenser opening. Switched ON the heating mantle and set the temperature at 60° C. After completion of 24 hours extraction, the heating mantle was switched OFF. The extract obtained, was dried in vacuum oven under vacuum to get it in powder form. This powder extract was then used for sample analysis.

Preparation of Eluent: - 0.477g of Sodium Carbonate and 0.067g of Sodium Bicarbonate was taken in 1000mL volumetric flask containing 500ml of ultrapure deionized water. It was swirled for 1 minutes and made up to the mark with ultrapure deionized water. It was then filtered through 0.2µ nylon membrane filter.

Preparation of standard solutions: -

Certified Sodium Bromide salt was procured from Merck. From this salt, a 1000mg/l standard Bromide solution was prepared. From this 1000mg/l standard solution, 0.05, 0.10, 1.00, 2.50 and 5.00 mg/l of Bromide was prepared for the Linearity study, and 1.0mg/l of Bromide was prepared for the precision study. 0.008mg/l of Bromide solution was prepared for Limit of Detection (LOD) and 0.028mg/l of Bromide was prepared for Limit of Quantification (LOQ).

Sample preparation: Around 0.1 g of Bacopa Monnieri and other herbal plant extract sample was weighed in 100ml polyethylene bottle with polyethylene cap, 50ml of ultra-pure deionized water was added to it. It was then mixed well after closing with cap. Samples were sonicated for 15mins and transferred it to centrifuge machine. It was centrifuged at 5000rpm for 10mins. Supernatant was filtered through 0.2u Nylon membrane filter. Samples were passed through OnGuard II RP (Thermo Fisher Scientific Dionex PN 057084) to remove organic matrices. It was collected in auto sampler vial. This procedure was repeated for each sample along with recovery samples and diluent. An Autosampler (Dionex AS-AP) was used to inject solutions containing Bromide into the ion chromatography system. Subsequently, the standard solution in the sample loop was transferred onto the separator column, on which bromide was separated. After separation on the column, Bromide was detected with suppressed conductivity detector. A sequence containing the blank, standards, samples and recovery samples were run, and results were then interpreted. Following is diagram which shows connections with various assembly of Ion Chromatography system

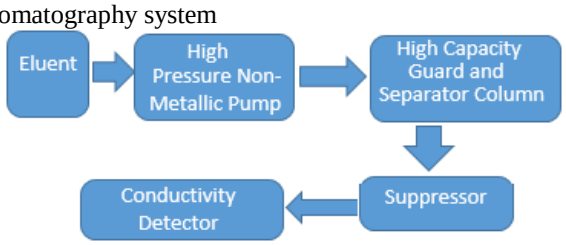

Figure 1: Ion Chromatography system schematic diagram for Bromide analysis

3. Results and Discussions

Limit of Detection (LOD) for Bromide was 0.008mg/l and it was injected (n) six times and observed average signal to noise ratio (S/N) was 3.2. Limit of Quantification (LOQ) for Bromide was 0.028mg/l, it was injected (n) six times and

observed signal to noise ratio (S/N) was 10.5. Table 1 shows results for LOD and LOQ of Bromide.

Table 1: LOD and LOQ data for Bromide

Iodate	*Amount, mg/l*	*S/N*	*% RSD (n=6)*
LOD	0.008	3.2	3.88
LOQ	0.028	10.5	1.65

The response of Iodate was linear over the range of 0.05 to 5.0mg/l of Bromide. Calibration curve fits well and that is significantly linear having correlation coefficient 0.9995 (figure 2). Each standard injection was repeated thrice. Therefore, number of calibration points (n) for linearity study was 15. Its data had been shown in table 2.

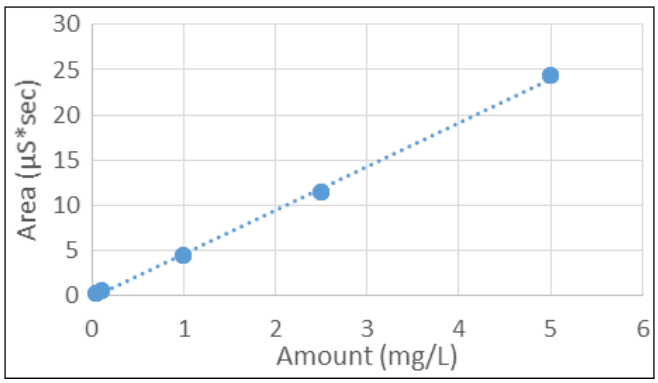

Figure 2: Linearity plot for Iodate and Bromate

Table 2: Linearity data for Bromide

Analyte	*Points*	*Corr. Coeff.*	*Offset*	*Slope*
Bromide	15	0.9995	0	0.079

Method specificity was also done with separate of Bromide (1.0mg/l) from mixture of other anions. Its chromatogram was shown in figure 3.

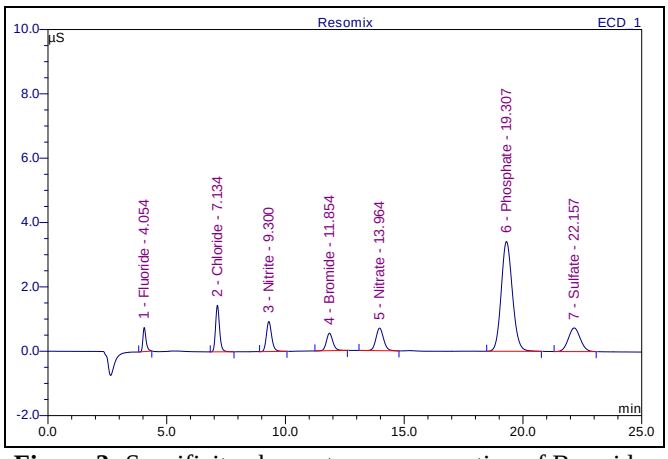

Figure 3: Specificity chromatogram separation of Bromide (1.0mg/l) from other anions

Replicate injections of Bromide were done and their percent relative standard deviation for peak area was 0.88%. Table 3 shows results for its precision study.

Table 3: Precision data for Bromide

Analyte	*Amount, mg/l*	*% RSD (n=6)*
Bromide	1.00	0.88

Chromatogram of Bromide standard injections is shown in figure 4.

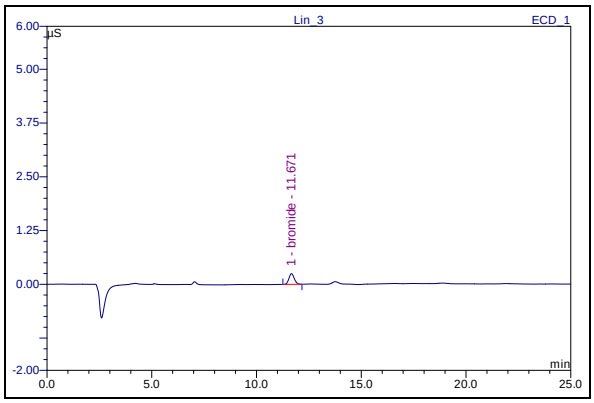

Figure 4: Standard chromatogram of Bromide (1.0mg/l)

Sample results: - Samples were analysed using the linearity calibration method. Replicate injections of same sample were also done. Its results and routine analysis sample results were shown in table 4 and table 5.

Table 4: Sample precision

Analyte	*Sample*	*Number of preparations*	*Results, mg/Kg*
Bromide	Bacopa Monnieri (Brahmi) (S. I. No. BM01)	10.0	85.63

Table 5: Routine sample analysis results

Sample	*Sample Identification Number (S.I. No.)*	*Bromide* mg/Kg
Bacopa Monnieri (Brahmi)	BM02	1273.99
Bacopa Monnieri (Brahmi)	BM03	569.95
Tulsi	TU01	152.59
Centella Asiatica	CA01	387.66
Salvia Officinalis	SO01	Not Detected
Mucuna Pruriens	MP01	Not Detected
Ashwagandha	AW01	76.05
Beetroot	BT01	36.14
Emblica Officinalis (Amla)	EO01	Not Detected
Pterocarpus Marsupium	PM01	Not Detected
Coleus Forskohii	CF01	Not Detected
Horse Chestnut	HC01	Not Detected
Pomegranate	PG01	Not Detected
Licorice Root	LR01	Not Detected
Boswellia Serrata	BS01	Not Detected
Garcinia Cambogi	GC01	15.13
Grape Seed	GS01	Not Detected
Rosemary	RM01	Not Detected
Fenugreek Seed	FS01	Not Detected
Ginger	GG01	Not Detected
Terminalia Bellerica	TB01	27.11
Phaseolus Vulgaris	PV01	Not Detected

Bromide was detected in some of samples, which indicates bromide intake by plant during their metabolism or it because of usage of Bromomethane (methyl bromide) as gas fumigant during their cultivation or storage.

Intraday analysis of Samples was done for seven consecutive days for which sample results were observed to be similar as given in table 5. Sample Chromatogram was shown is figure 5.

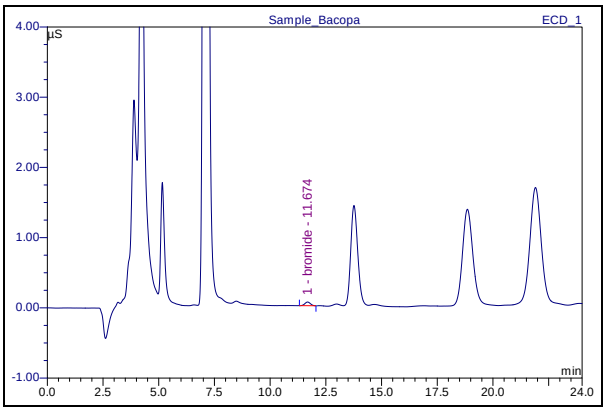

Figure 5: Sample chromatogram of Bacopa Monnieri (Brahmi) (S. I. No. BM01)

Recovery: The sample used for recovery study was Bacopa Monnieri (Brahmi) (S.I. No. BM01) (average concentration was taken for calculation). Recovery test solutions were injected in triplicate Also for recovery study, known concentrations of amount was added to sample at three different levels as shown in table 6.

Table 6: Recovery study (Bromide) for sample (Bacopa Monnieri (Brahmi) (S.I. No. BM01) (n = 3)

Analyte	*Level*	*Amount Added* mg/l	*Amount Recovered* mg/l	*% Recovery*
Bromide	1	0.050	0.048	96.10
	2	0.500	0.486	97.20
	3	1.000	0.980	98.00
	4	2.500	2.589	103.56

The same method has been used on another Ion chromatography model like ICS 5000+ and ICS Integrion with different lot of IonPac AS23 column as a part of ruggedness study, for which there is no significant variations of sample results were observed

4. Conclusions

Ion Chromatography with suppressed conductivity detection gives specific, sensitive and precise method for estimation of Bromide from various herbal plants samples. This present method was used for analysis of plant samples for Bromide content with easier pretreatment process. The detection limits for Bromide was 0.008mg/l. This technique is cost-effective with respect to analysis required for keeping a check on the limits of Bromide for which it can be included in regulatory bodies to keep check on quality of Herbal and Ayurvedic medicines

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Author Profile

Chetan Chavan had completed B.Sc (Chemistry). and M.Sc (Chemistry) from Mumbai University and completed his Ph.D in year, 2018., He had joined Dionex India Pvt. Limited, which is now Thermo Fisher Scientific Pvt. Ltd., as Applications Manger for Ion Chromatography (IC) and HPLC and currently working as Product Manager – IC/SP. His role is to provide technical support coordination with factory for India team

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Chanakya Thaker received the B. Sc (Chemistry). and M.Sc (Analytical Chemistry) degrees from Mumbai University in 2006 and 2008, respectively. He had received Honorary Doctorate in Life Sciences from CCU/USA in 2019. He had completed MBA-Marketing in 2019 from ITM Vocational University. From 2009, he had joined Dionex India Pvt. Limited, which is now Thermo Fisher Scientific Pvt. Ltd., as Applications Specialist for Ion Chromatography (IC) and HPLC and currently working as Applications Manager – IC/SP. His role is to manage application team, provide customer training, sample analysis, technical presentations, method development and troubleshooting on IC and HPLC systems.

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