

**DETERMINATION OF BORON IN NUCLEAR MATERIALS AT SUBPPM LEVELS BY
HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)**

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ABSTRACT

Experiments were conducted for the determination of boron in U_3O_8 powder, aluminium metal and milliQ water using dynamically modified Reversed Phase High Pressure Liquid Chromatography (RP-HPLC) and using two precolumn chromogenic agents viz. chromotropic acid and curcumin for complexing boron. The complex was separated from the excess of reagent and determined by HPLC. When present in subppm levels, chromotropic acid was used successfully only for determination boron in water samples. For determination of boron at subppm levels in uranium and aluminium samples, curcumin was used as the precolumn chromogenic agent. The boron curcumin complex (rosocyanin) was formed after extraction of boron with 2-ethyl-1,3-hexane diol (EHD). The rosocyanin complex was then separated from excess curcumin by displacement chromatography. Linear calibration curves for boron amounts in the range of 0.02 μg to 0.5 μg were developed with correlation coefficients varying from 0.997 to 0.999 and were used for the determination of boron in aluminium and uranium samples. Precision of about 10% was achieved in samples containing less than 1 ppmw of boron. Detection limit of this method is 0.01 μg boron.

1. INTRODUCTION

Determination of boron at subppm levels in materials such as uranium metal, uranium oxide fuels and aluminium clad is an important requirement in the nuclear technology. Emission spectroscopy, spectrophotometry and mass spectrometry methods with detection limit of about 0.1 ppmw of boron, are available in literature. For most of the spectrophotometric methods, errors in the determination arise due to two reasons:

(i) The chromogenic reagents and the boron chromogenic complex absorb at the same wavelength, which degrades the detection limits. This becomes significant when trace quantities of boron are to be determined, (ii) Absorbance due to interfering species present in the solution.

A method where the reagent and the complex can be separated would be more reliable. HPLC technique can be used for separation of nanogram amounts of boron as a boron complex. Another advantage of HPLC is that any other interfering element, if present, is also separated from the complex during elution. Experiments were carried out using both chromotropic acid and curcumin commonly used in spectrophotometric methods for complexing with boron. Separation conditions for both were studied, the details of which are described below. Chromotropic acid was initially used because of the ease of complex formation and the stability of the complex over a wide pH range of 2-8. This method has also been reported in literature [1,2].

2. DIFFERENT METHODOLOGIES REPORTED

Boron has been a difficult element to analyse especially when present in subppm amounts due to several reasons such as (i) availability of suitable and well characterized reference materials, (ii) loss of boron during dissolution of sample, (iii) easy contamination of sample with boron present in environment and (iv) commercial availability of instrumental methods for analysis of boron at subppm levels which would make low boron blanks go undetected causing error in analysis.

Different methods like colorimetry [3-11], atomic emission spectrometry [12-14], mass spectrometry [15,16], as well as non-destructive methods like nuclear track detection or prompt gamma neutron activation analysis (PGNAA) [17,18] have been reported for the determination of boron. Almost all methods, except the non-destructive methods, require an initial separation of boron from matrix and a preconcentration step. Spectrophotometric methods using derivatives of anthraquinone such as dianthrimide, quinalizarin, carminic acid, 1,8-dihydroxyanthraquinone have been used for complexing with boron but the complex is formed only in concentrated sulphuric acid medium and in some cases, final concentration of sulphuric acid is critical. Chromogenic reagents such as curcumin, chromotropic acid, azomethine H are being used more often. In the curcumin method used widely, boron is determined spectrophotometrically after separation by distillation as methylborate [3]. More recently, 2-ethyl-1,3-hexane diol (EHD) has been used for extraction of boron into an organic medium such as chloroform after which the curcumin method is used [4-7]. Advantage of using EHD is that boron is present in non-aqueous medium instead of water, which interferes with color development with curcumin if more than 0.2 mL is present. The boron was extracted from different dissolution media like 2M HCl for boron in ashed animal tissues [5], 1M sulphuric acid for boron in steel [6], dilute sulphuric acid for boron in Uranium Oxide [7]. In all the cases, boron was found to be completely extracted. 10% (0.65M) EHD in chloroform was used by most of the analysts for boron extraction, and 0.375% (0.01M) curcumin in acetic acid for color development. Chromotropic acid is also another reagent which has been used for the determination of boron spectrophotometrically. The boron chromotropic acid complex was extracted using Zephiramine into 1,2-dichloroethane and absorbance of the organic phase was measured for determination of trace amounts of boron in natural water [11]. A method was reported [10] for determination of boron based on fluorescence intensity of boron complex with chromotropic acid in aqueous solution. Kiss [19] used boron specific resin Amberlite XE-243 for preconcentration of microgram amounts of boron present in rock, after fusing the rock or mineral sample with potassium carbonate for its dissolution. Spectrophotometric method with carminic and azomethine H was used for determination of boron. Agazzi [12] used flame emission technique for determination of boron after extraction of boron with 2% to 10% EHD in chloroform. Boron was completely extracted with a single extraction of 5 minutes when aqueous to organic ratio was two. Muzzarelli [20] used ion exchange chromatography where uranium nitrate was dissolved in ethylether and loaded onto an anion exchange column conditioned with ethyl ether. Uranium was not held whereas boron was retained, and was washed with 5N HCl. Boron recovered was then determined by emission spectrographic method. In the recent years, for boron analysis by ICP-AES, organic extractants have been used to preferentially extract uranium leaving boron in aqueous

phase[14]. A negative thermal ionization mass spectrometric technique was used to analyse boron in traces in metals and alloys by IDMS where the BO_2^- ions were monitored [15]. Distillation of methyl borate ester was used for selective separation of boron after metal was dissolved in acids. Boron in aluminium, zirconium and steel were determined by this method. Boron was determined at ppm and subppm levels with standard deviation of 7% to 15%. Online ICP-MS [16] technique has also been used for determination of B in sodium and uranium. Cation exchange resin was used online for Na matrix removal and TOPO was used for selective extraction of uranium prior to analysis. Detection limit was 0.2 ppm of B in UO_2 and 0.1 ppm of B in Na.

In the recent years, HPLC techniques with online absorbance detectors have been used for determination of the separated boron-complex. Ion-pair liquid chromatography and ion exchange chromatography have been used for separation of boron chromotropic acid complex from excess of reagent [1,2]. Using this method, boron in steel and water have been determined. Trace amounts of boron in water were determined by ion-exclusion chromatography with postcolumn method using azomethine H as chromogenic reagent [21].

The aim of the present work was to develop HPLC method for determination of subppm amounts of boron in uranium and aluminium samples. As mentioned earlier, the merit of HPLC technique vis a vis a spectrophotometry is primarily because of removal of interferences thus making it a reliable and sensitive method of analyses.

3. INSTRUMENTATION

A schematic of the HPLC system used in the present work is given in Fig.1. It consists of an L-7100 (Merck Hitachi) Quadratic gradient pump, a Rheodyne sampling valve (Model 7725i) with 20 μL sample loop, a guard column C_{18} type (1cm), a Chrompak C_{18} type analytical column (15cm x 0.4cm) with 5 μm particle size, a column oven L-7350 (which houses the injector, guard column and the analytical column) and a 512 bit Diode Array Detector (DAD) L-7450A (Merck Hitachi) which measures the absorbance from 200nm to 800nm. The eluted species flowing through the 10 μL flow cell are monitored at specified wavelengths defined in the method. The signal from the detector is processed by HSM D-7000 software package and the chromatograms are recorded using PC. The HPLC system was computer controlled through interface D-7000 (Merck Hitachi).

Quartz ware was used for dissolving the samples. Extractions were carried out in polypropylene tubes. All apparatus and flasks were thoroughly leached in 3M nitric acid, washed with milliQ water and methanol, and dried before use. For transferring solutions, variable volume pipettor with disposable tips was used.

4. EXPERIMENTAL

4.1 Separation and determination of boron as boron chromotropic acid complex by RP-HPLC

4.1.1 Reagents used

Chromotropic acid (Fluka;Switzerland), EDTA (E. Merck;India), Tetrabutylammonium bromide (Fluka;Switzerland), Fresh deionised water with resistivity of 18 M Ω .cm, High purity methanol (99.8% purity) (E. Merck;India). The mobile phases used were filtered through 0.45 μ m membrane filters (Millipore).

4.1.2 Procedure

Boric acid forms a reasonably quick and stable anionic complex with chromotropic acid [i.e 1,8 dihydroxy naphthalene 3,6 disulfonic acid] of the type $[B(O_2R)_2]^{-5}$ [2]. The complex is stable over a wide pH range and has a number of absorbance maxima in the 315-370 nm range similar to that of chromotropic acid. Studies for HPLC separation of the complex from the reagent using bonded phase column of C₁₈ type by ion pair phase chromatography were carried out. Tetrabutylammonium bromide (TBA⁺Br⁻) was used for ion pairing with the anionic complex to form the neutral entity, which is retained on the column. The different aspects of the studies are discussed below.

Formation of the complex: To a solution containing upto 10 μ g of boron, 0.2 mL of 0.15M aqueous solution of chromotropic acid and 0.2 mL of 1M TBA⁺Br⁻ solution was added. 0.4 mL of 0.1M EDTA solution was added for masking interfering ions. The solution was kept in the dark for 15 min to allow complete complex formation. This solution was diluted to 5mL and 20 μ L of the diluted solution was injected into the column.

Optimisation of elution condition: Initially when novapak column(15cmx0.4cm) with particle size of 4 μ m was used, gradient elution was required(0-3.2min, 0.01M TBA⁺Br⁻ in 55% methanol, 3.2-3.5min 0.01M TBA⁺Br⁻ in 65% methanol till 15min) as broadening and tailing for the reagent and boron-complex peak was observed when isocratic mode was used. Using the higher strength of mobile phase initially gave poor resolution for the reagent and complex peaks. The retention times of the reagent and boron complex were 2.1 and 7.0 minutes, respectively. However when the column was replaced with chropak column of 15cm length and particle size of 5 μ m, isocratic mode of elution with 60% methanol and 0.0075M TBA⁺Br⁻ was used. The complex was found to elute corresponding to retention time (RT) of 5.1 min. The reagent elutes at a RT of 2.8 minutes. Chromatograms were recorded at 350 nm using the online diode array detector. It was found that peak response at lower wavelengths (250nm-200nm) was much higher than at 350nm. However the peak response was not very linear and seems to be critically dependant on the molarity of TBA⁺Br⁻ and hence was not considered useful for analytical purposes.

Calibration curve: A calibration curve was obtained for boron amounts ranging from 0.1 μ g to 2.5 μ g. Peak area was used for quantification. The reproducibility was found to be

better than 5%. The calibration plot is shown in Fig.2. The boron amount on the X axis is the amount initially taken, the amount actually injected into the column ranges accordingly from 0.4 ng to 10 ng.

4.1.3 Results and Discussion

Boron in water: Using this method, boron in milliQ water and tap water was determined by HPLC. Varying quantities of water from 100mL to 10mL were taken in quartz beakers and evaporated to near dryness under IR lamp. A few drops of NaOH (1M) were added to prevent evaporation of boric acid in the samples. Blank readings were also taken. Boron concentration was found to vary from 0.0025 $\mu\text{g}/\text{mL}$ to 0.0097 $\mu\text{g}/\text{mL}$ in milliQ water and 0.014 $\mu\text{g}/\text{mL}$ to 0.030 $\mu\text{g}/\text{mL}$ in tap water (Table I). It appears that varying amounts of boron in tap water affects boron amounts in milliQ water.

Boron in Aluminium and Uranium: Further experiments were carried out to check the feasibility of boron determination at subppm levels in uranium and aluminium. Both metals form complexes with chromotropic acid. Complexation was carried out in the presence of 10 to 200 mg (0.005M to 0.1M) of uranium spiked with boron amounts ranging from 0.2 to 0.5 μg . The pH was carefully raised to 2.5 with dilute ammonia taking care to see that precipitation of uranium due to hydroxide does not occur. 0.4mL of chromotropic acid solution, 0.4mL of TBA⁺Br and 0.4ml of EDTA were added and the solution was diluted to 10mL. 20 μL of this solution was injected into the HPLC column. It was found that the response for boron complex was less than expected for all solutions. When the pH of the final solution was less than 2, no peak for boron appeared. Masking of concentrated solutions (10^{-1}M) of the matrix element with EDTA or with any other masking agent is difficult as solubility product is exceeded resulting in precipitation. Similarly for aluminium it was observed that for upto 5 mg of aluminium containing about 0.2 μg boron, response for the boron complex could be obtained. At 10 mg level, it is difficult to maintain the required pH condition without precipitation of aluminium. Two factors contribute to inhibit boron complex formation a) Difficulty in adjusting pH conditions suitable for boron complex without precipitation of hydroxide and b) Competition from U and Al which also complex with chromotropic acid. Therefore, the matrix element has to be masked using a suitable masking reagent without allowing the solubility product to exceed under the experimental conditions. However scope for using this method for samples containing more than 10 ppmw of boron still exists as boron can then be determined from dilute sample solutions. A preliminary separation step was, therefore, essential when subppm levels of boron in metal or metal oxides has to be determined. Established methods such as methyl borate distillation procedures are slow and tedious, require special apparatus, and are prone to boron losses. A quick and highly efficient method (100% extraction) with minimum losses consists of extraction of boron by 2ethyl-1,3-hexane diol(EHD) [4-7,12]. Boron present in an acidic solution forms a weak chelate with EHD which is soluble in chloroform. However, our experiments showed that chromotropic acid did not form a complex with boron present in the organic extractant. On the other hand, aqueous based separations like ion-exchange methods are not as efficient and would result in high blank values because of high volumes of solutions required for washings. Therefore, it was thought advisable to change the color forming agent to curcumin. The draw back while using curcumin is that the complex is

formed at high acidity [0.5mL of concentrated (18M) sulphuric acid diluted with ethanol to give a 0.35M solution prior to spectrophotometric measurement] which is not a very desirable operating condition for the HPLC column. However, during our experiments, we found that 0.25mL of sulphuric acid is sufficient for complex formation and gives reproducible absorbance values. Hundred fold dilution reduces the acidity to less than 0.1N. Though the dilution step destroys part of the excess protonated curcumin, (which absorbs at same wavelength as that of the boron complex), the contribution to the absorbance of nanogram amounts of boron complex is substantial and can, therefore give erroneous results. Even at this dilution, the highly sensitive DAD detector could detect and measure the absorbance of the separated boron complex (rosocyanin) from excess reagent. The experimental details are given below.

4.2 Separation and determination of boron as boron curcumin complex (rosocyanin) from excess of reagent

4.2.1 Reagents used

2-ethyl-1,3-hexanediol (EHD) (Merck;Germany), Curcumin (Ferak;Germany), High purity sulphuric acid (Scharlau;Czech Republic), Tetrabutyl ammonium bromide (Fluka; switzerland), High purity methanol (E.Merck;India)

4.2.2 Procedure

Since both the protonated curcumin and rosocyanin complex absorb at 550 nm, separation of excess reagent from the complex by HPLC is desirable as it would improve the detection limit and accuracy of results. This complex is formed after extraction of boron into organic medium by EHD. Various studies undertaken to develop the procedure were as follows.

Extraction procedure: Procedure developed was based on the earlier methods reported in literature [4-7]. Extraction of boron was carried out from 10mL of 1M HCl solution by 10% (0.65M) EHD in chloroform. The extraction was carried out in two batches of 2 mL and 1mL of extractant for a duration of 3 minutes each. In addition, washing of solution with chloroform was also done. Polypropylene test tube with leak proof cover was used for all extractions. All extracts collected were combined in quartz beaker. This method gave 100% extraction for boron. When the third extract was tested for boron, absorption values similar to those for blank values were obtained suggesting that all boron is extracted completely in the first two extractions. Chloroform in the boron extract was evaporated off by heating the extract under IR lamp.

Complex formation.: The boron complex, rosocyanin, is formed by reaction of protonated curcumin with boron; there is a dispute about whether the ratio of reagent to boron in the complex is 3:1 or 2:1 [3]. The rosocyanin complex was formed by addition of 1mL of 0.01M curcumin in acetic acid and 0.25mL of 18M sulphuric acid to the extract. The addition of 0.25mL of sulphuric acid was found sufficient for giving reproducible absorbance values. After 30 minutes, the solution was diluted with methanol to 10 mL. To 0.5 mL of this solution, 0.3mL of 0.05M TBA⁺Br⁻ was added and the solution was further diluted to 5mL with methanol. This dilution step lowers the

acidity to less than 0.1N and destroys a part of the protonated curcumin. 20 μ L of this solution was loaded onto the HPLC column.

Mobile phase studies: Initially, when 85% methanol was used as the mobile phase, chromatogram for curcumin and its protonated species was obtained and the boron complex was found to be retained on the column. It appeared that though the complex is ionic, its large hydrophobic group is strongly held on the nonpolar C-18 column and thus is not eluted by methanol. When 0.003M n-octane sulphonate was added, a broad peak of the complex was obtained at 4 minutes for a flow rate of 1mL/min(Fig.3). The elution is caused by displacement action of n-octane sulphonate which displaces the boron complex (rosocyanin) adsorbed on the column. On replacement of octane group with a bulkier tetrabutyl group (by using 0.003M tetrabutyl ammonium bromide in 85% methanol), a much sharper peak at 2.9 minutes was obtained (Fig.4). The peak shape improved further when the elution was carried out at 35 $^{\circ}$ C in the column oven (Fig.5). Fig.6 shows the chromatogram of unprotonated curcumin. Fig.7 shows the chromatogram of the reagent blank and it shows different species eluting at the monitoring wavelength of 550nm. As can be seen, both the protonated curcumin and unprotonated curcumin contribute to the absorbance at 550nm.

4.2.3 Results and Discussions

The effect of moisture and EHD on the absorbance. Hayes and Metcalfe[3] have reported that presence of moisture in excess of 0.25mL can have an effect on formation of rosocyanin because of the lowering of acidity. In colorimetric determination of boron Donaldson [6] has used only a small portion of extract containing 0.1mL of EHD for color development to avoid interference from EHD. However, increasing the amount of extract would increase sensitivity making it applicable for samples with low boron content. Therefore, preliminary spectrophotometric studies were carried out to see if there was a change in absorptivity due to interference from moisture and EHD. Boron contained in different aqueous aliquot sizes was complexed with 1mL of curcumin and 0.5mL of sulphuric acid and was diluted to 25 mL with ethanol after half an hour. The observations as can be seen from the Table II are as follows (a) when the moisture content was above 0.3mL, the absorptivity is reduced. (b) Using 0.25mL sulphuric acid instead of 0.5 mL, reduces the absorbance slightly (c) 3mL of EHD in chloroform added to 0.1mL aqueous aliquot containing boron reduces the absorbance drastically (d) However, if this solution is heated under IR to remove chloroform and moisture, the absorbtivity increases. The EHD and Boron EHD complex formed have low volatility because of which there is no loss of boron even if heated under IR for as long as an hour. (e) There is no significant difference in absorbtivity between 1mL portion of extract containing 0.1mL EHD and 3mL portion of extract containing 0.3mL EHD. For 3mL extracts, the solution was heated under IR lamp to remove moisture and chloroform. Since there was no significant lowering of absorptivity, further work was carried out using all 3mL extract for colour development.

Development of calibration curve: Aliquots with boron in the range of 0.02 μ g to 0.5 μ g were used for developing the calibration curve (Fig.8). Peak height was used for quantification in view of the low concentration. For color development with curcumin, the water content in the aliquots should be as low as possible. Studies in our laboratory have shown that if the volume of solution containing boron is restricted to less than 0.2 mL, extraction of boron into organic phase according to the above mentioned procedure is not required for pure boron. This would simplify the procedure for obtaining the calibration graph considerably. To the aqueous solution (<0.2mL) containing boron, 3mL of 10%EHD in chloroform is added and the quartz beaker is heated under IR to remove chloroform. Addition of 3mL EHD was done to maintain the same condition as that of the extracted boron in actual samples. Complexation and HPLC separation were carried out according to the procedure described above. Reagent blank was also determined under identical conditions. Response was monitored in terms of peak height. The calibration curve was developed after subtraction of reagent blank response from the individual sample peak response. Also to ensure that the response of extracted boron is the same as that of directly complexed boron, known amounts of boron were extracted from 10mL of 1M HCl solution, complexed and separated, and from the peak response, boron was determined using the calibration curve developed as above using directly complexed boron (Fig.8). The estimated boron was found to be in good agreement with actual amount (Table III). This substantiates the earlier observation that the boron extraction is quantitative. For easy calculations when analysing samples, the boron amount (μ g) initially taken for complexing is used in the plots. The actual amount injected and thus loaded on the column varies accordingly from 4 picogram to 100 picogram.

Boron in U and Al standards: 5 synthetically prepared samples of working standards of uranium oxide containing boron in the range of 0.25 to 1.6 ppmw of boron and two aluminium standard samples from Indal and Aviatube of France containing less than 10 ppmw of boron were analysed. 100 to 500 mg of uranium oxide samples were dissolved in 15 mL of 3M nitric acid, evaporated to near dryness and subsequently dissolved in 10mL of 1M HCl. This was done as nitrate interferes in complex formation. Treatment was carried out under IR lamp so as to avoid any loss due to volatilization of boron [22,23]. For aluminium 80 to 120 mg of the standards were dissolved in 10mL of 2M HCl. Boron was extracted from the 1M HCl solution by EHD, complexed with curcumin and subjected to HPLC separation according to the procedure detailed above. To find out if there is boron loss during dissolution, uranium free from boron was spiked with known amounts of boron and subjected to dissolution, evaporation and analysis. The amount determined was in very good agreement with the actual value confirming that boron is not lost during the dissolution step (see Table III). It appears that in presence of matrix, boron does not volatilise so easily. Reagent blank was also determined under similar conditions. As heating the acid alone could result in loss of boron [22,23] and thus lead to erroneous value for blank, about 5 milligram of boron free uranium/aluminium was added to the dissolving acids during the evaporation step. This helped to give accurate values for reagent blank. The reagent blank determined includes boron in the dissolution acids and extraction medium (10mL of 1M HCl).

Table IV gives the range of reagent blank values obtained during the period of analysis. The values marked with(*) are boron values determined in duplicate on the same day to check for the reproducibility of reagent blank, as accurate determination of the blank is important for obtaining accurate boron values in the sample. The last row of the Table gives the boron obtained in extraction medium only (10mL of 1M HCl). Table V gives the values of boron obtained as against the expected values. Two independent values given for each sample were obtained from separate experiments. There is a fairly good agreement in the boron values obtained by HPLC method vis-à-vis the expected values arrived at by different analytical methods like ES, SSMS and spectrophotometry. It should also be mentioned that there is large uncertainty in the expected values which are based on mean of means of values obtained by different methods used in 1982. However, the values obtained using HPLC are in good agreement for each of the three ILCE samples. For CRM samples prepared and characterised in 1999, there is excellent agreement between the values determined by HPLC and the expected values. For the two aluminium samples, the expected values are not certified but are only indicated for information purpose. The duplicate values of boron for each sample obtained on the same day are in good agreement.

Hence the above studies demonstrate the applicability of HPLC method for determination of boron at subppm levels in uranium and aluminium samples.

5. CONCLUSION

Analysis of boron by HPLC using curcumin as a chromogenic agent is a very sensitive technique. Boron at subppm levels can be determined with a reproducibility of better than 10% in samples like uranium and aluminium.

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Table I

Boron in tap water and millipore water

S. No.	Boron in Tap water($\mu\text{g/mL}$)	Boron in Millipore water ($\mu\text{g/mL}$)	Aliquot size
1	0.0298	-	10 mL
2	0.0138	0.0023,0.0096	50 mL
3	0.0150	0.0025	100mL

Table II

Effect of moisture and EHD on absorbance of rosocyanin

S No.	Sample type containing boron	Absorptivity of the complex ($\text{L.mole}^{-1}.\text{cm}^{-1}$)
1	0.15mL aqueous solution	1.73×10^5
2	0.35 mL aqueous solution	1.31×10^5
3	0.15 aqueous solution(0.25mL H_2SO_4)	1.51×10^5
4	0.1mL aliquot in 3mL of 0.65M EHD in chloroform	absorbance less than blank
5	0.1mL aqueous aliquot evaporated under IR lamp after addition of 3ml of 0.65M EHD in chloroform	1.6×10^5
6	0.4mL aqueous aliquot evaporated under IR lamp after addition of 3 mL of 0.65M EHD in chloroform	4.06×10^3
7	1mL portion of 2.5mL EHD extract unevaporated	1.58×10^5
8	3 mL extract evaporated under IR lamp	1.55×10^5

Table III

Standard boron solution extracted and determined using the calibration curve of directly complexed boron

S. No.	Sample	B determined (μg)	Expected value (μg)	% difference
1	Boron Std extracted	0.137	0.128	+6.9
2	Boron Std extracted	0.257	0.253	+2
3	Boron Std extracted	0.403	0.396	+1.7
4	U free from B, spiked with boron	0.078	0.077	+1.2

Table IV

The range of Boron values in Reagent blank

S. No.	Reagent type	Boron in μg
1	Dissln soln for U (15mL of 3M HNO_3) +extn med	0.035 - 0.050
2	Dissln soln for Al (10mL of 2M HCl) +extn med	0.056- (0.069/0.064)*
3	Extn medium(1M HCl in 10 mL milliQ water)	(0.019/0.014)*-0.029

* 2 blanks determined on the same day

Table V

Determination of Boron in Uranium and Aluminium samples

S. No.	Sample	B amt in ppmw (Results of duplicate analysis)		Expected value**	Sample size (mg)
1	ILCE I(U ₃ O ₈)	0.104	0.111	0.21±30%	500
2	ILCE II(U ₃ O ₈)	0.211	0.206	0.32±43%	500
3	ILCE III(U ₃ O ₈)	0.566	0.554	0.73±32%	500
4	CRM-1(U ₃ O ₈)	1.02	1.09	1±30%	200
5	CRM-2(U ₃ O ₈)	1.60	1.70	1.6±19%	100
6	Al (INDAL)	8.32	8.40	< 10 ppm	100
7	Al(French)	6.65	6.72	< 10 ppm	100

** Average of Inter Laboratory Comparison Experimental(ILCE) values for S.Nos. 1 to 5

Note: ILCE experiments were carried out in 1982 by FCD employing different methods such as ES, SSMS,AAS,spectrophotometry among different DAE laboratories in India. Certified Reference Material, CRM 1,2[24] were prepared and characterised in 1999 in DAE employing ICP-AES, DC-arc-AES, ICP-MS techniques.

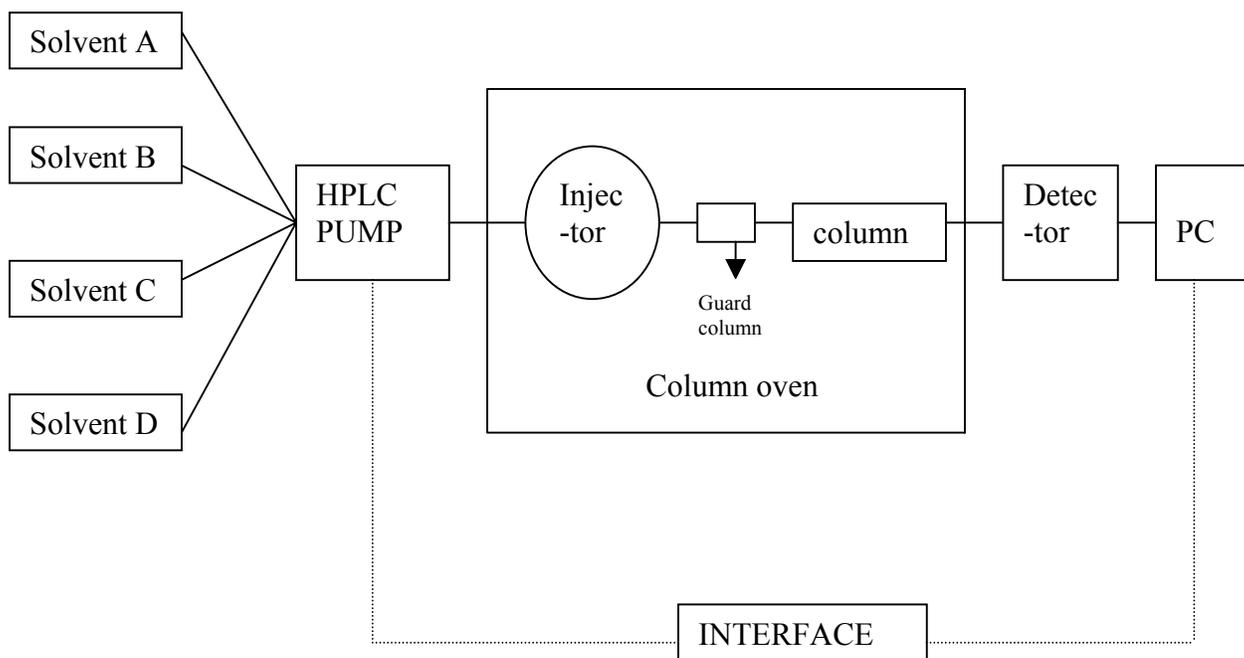


Fig 1. Schematic diagram of the HPLC system with quadratic low pressure gradient pump unit attached with an online diode array detector system.

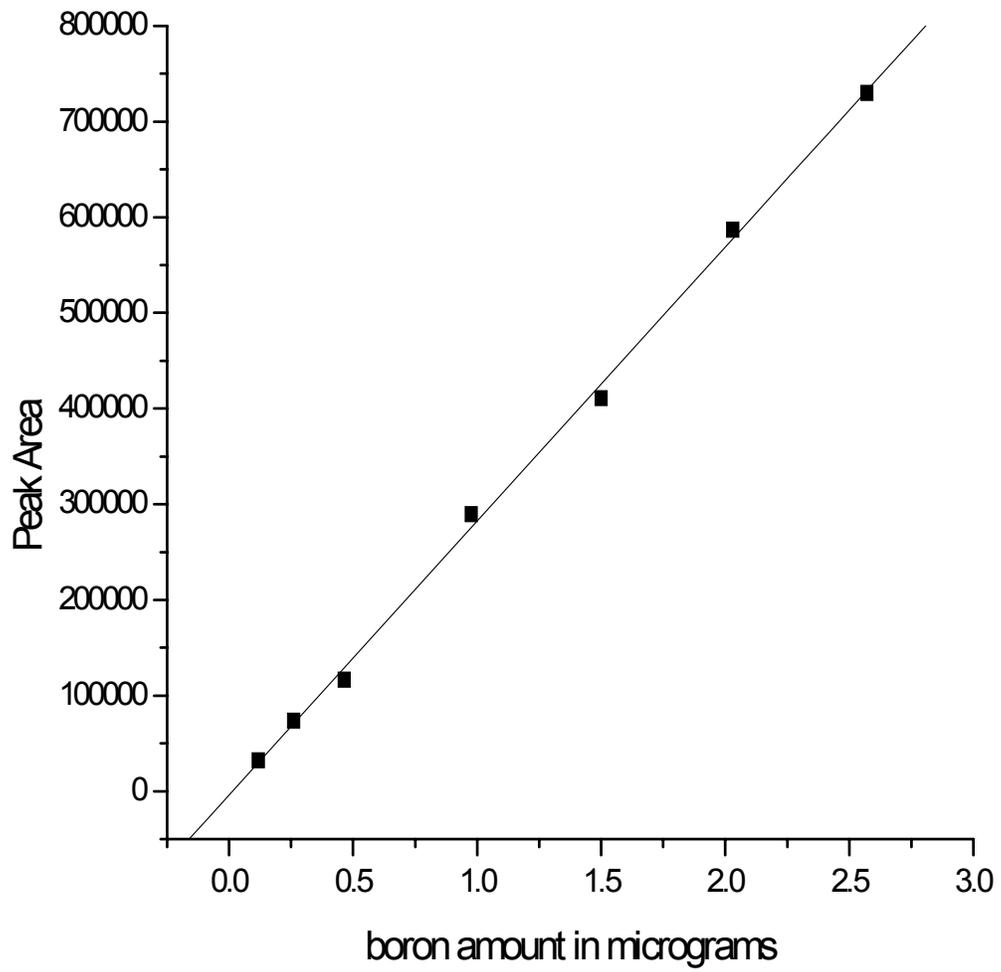


Fig 2. Calibration plot obtained for boron-chromotropic acid complex. 20 μL of 1×10^{-6} M to 2.5×10^{-5} M solution loaded into the column.

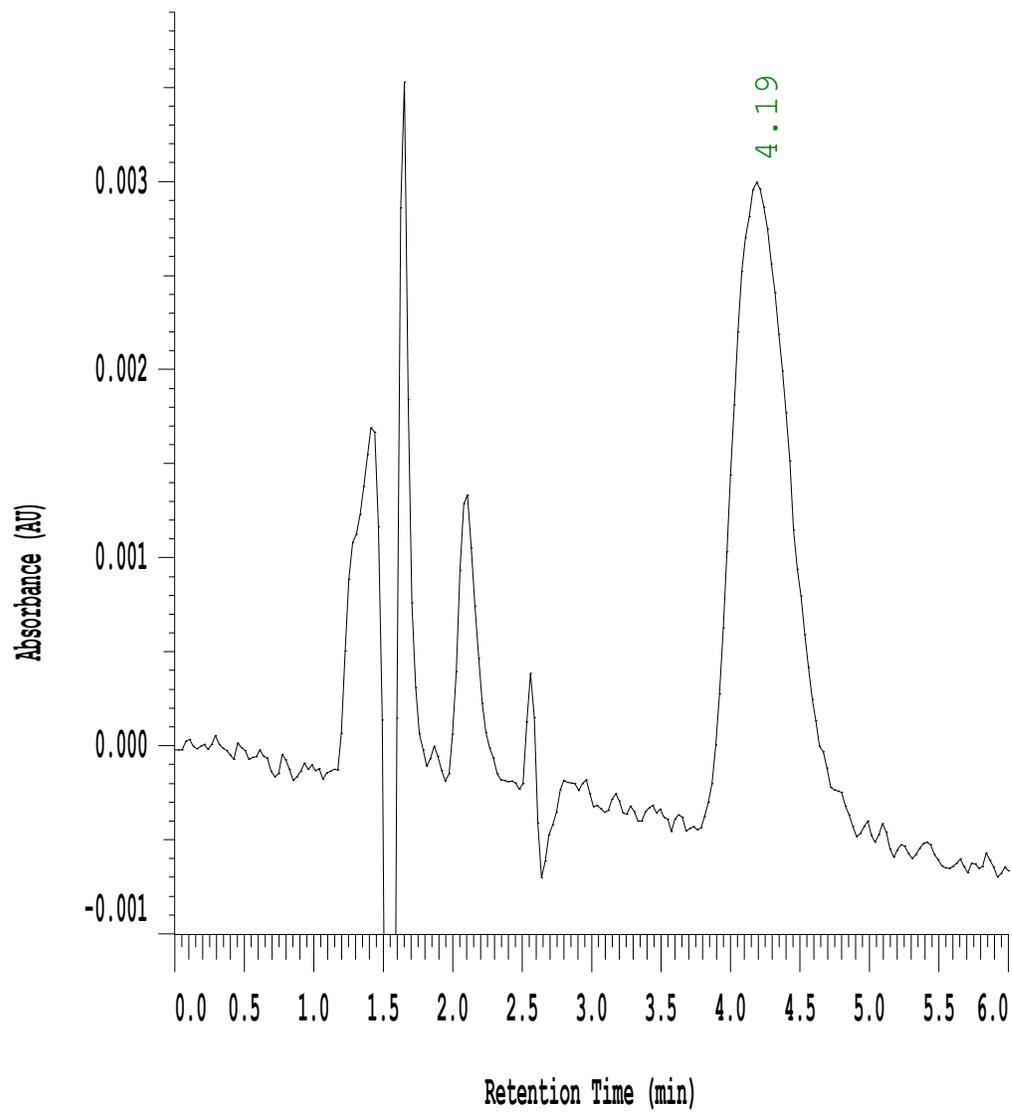


Fig 3 Mobile Phase: 0.003M n-octane sulphonate in 85% MeOH. Flow rate:1mL/min, boron injected 100 picogram, R.T. 4.2 min, monitoring wavelength-550nm.

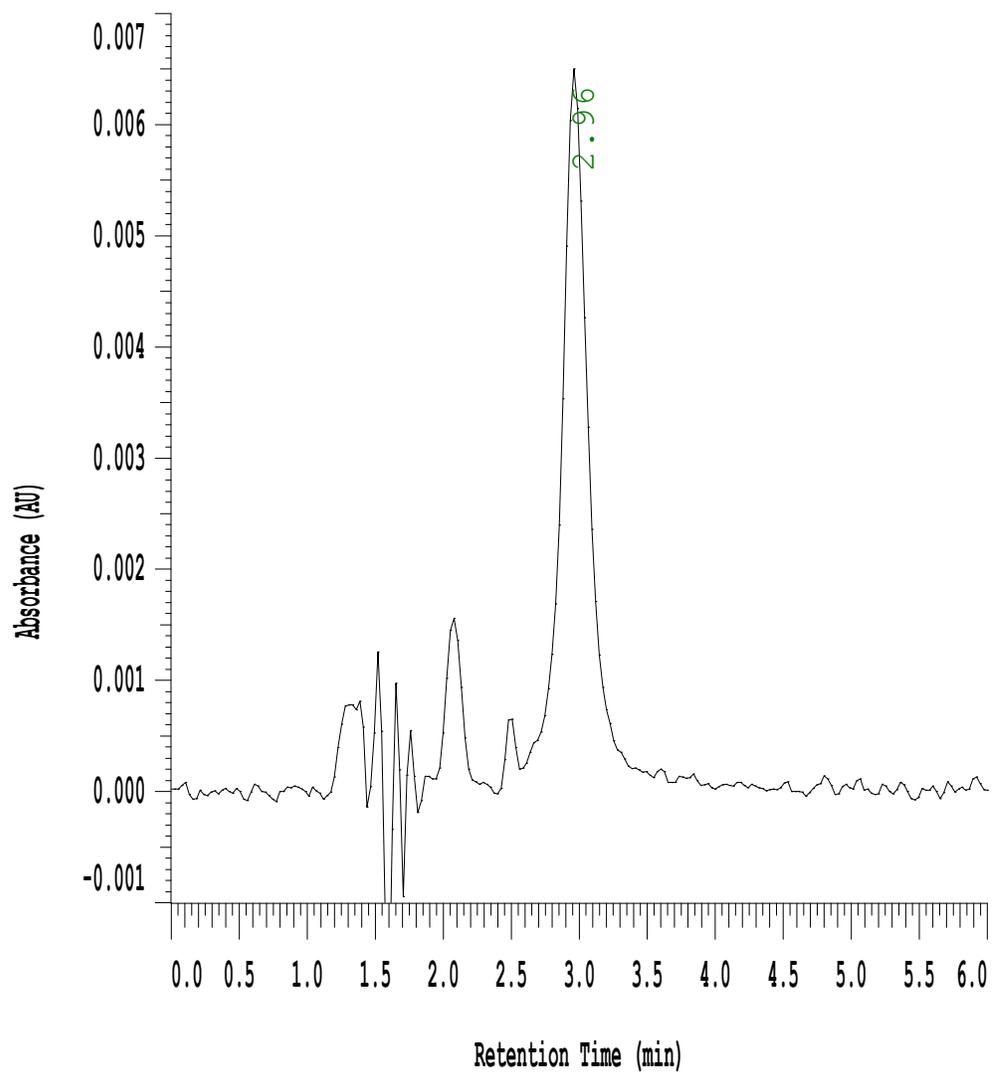


Fig 4 Mobile Phase : 0.003M TBABr in 85% MeOH, results in sharper peak for the complex at 2.96minutes. F.R. 1 mL/min, boron amount injected 100 picograms, monitoring wavelength -550nm

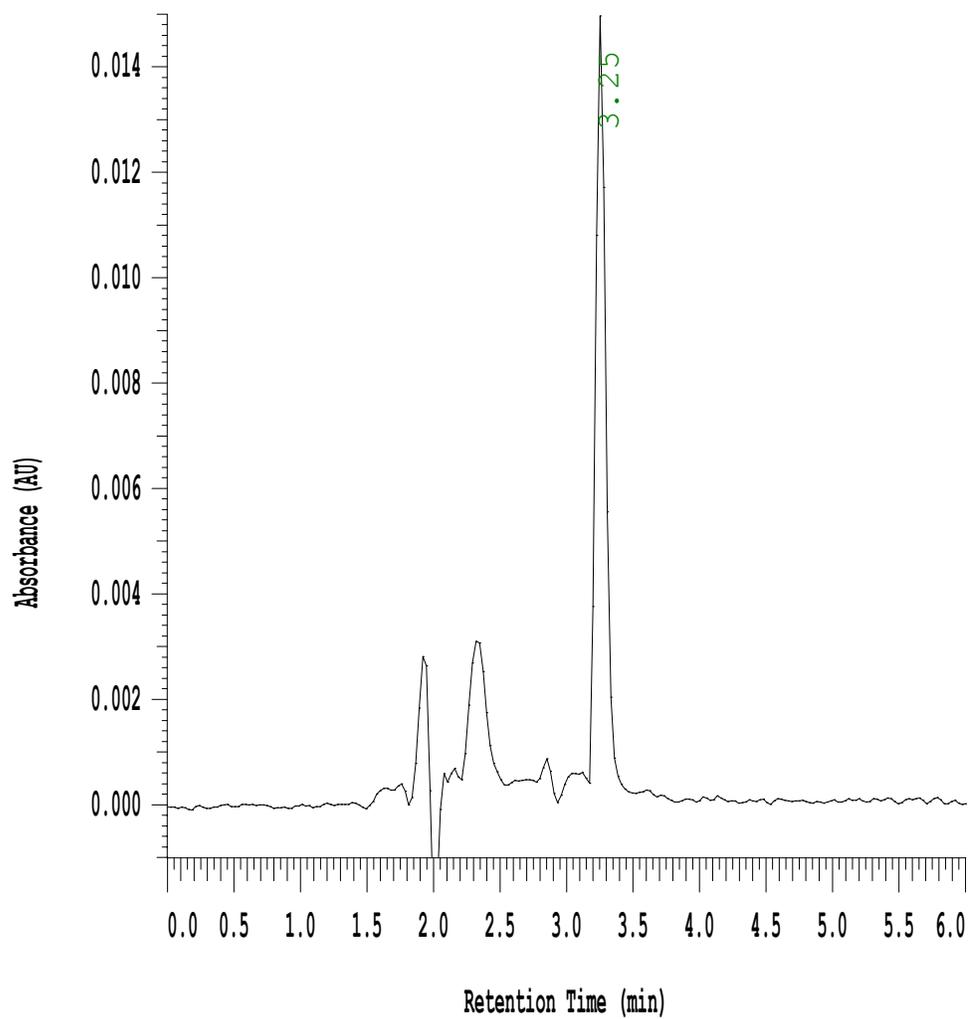


Fig 5 Chromatogram under optimized condition. Mobile Phase 0.003M TBABr in 86% methanol. Isocratic mode of elution. Flow Rate 0.8mL/min, Column oven temperature 35°C, Monitoring wavelength 550nm, Boron loaded on column 100 picogram.

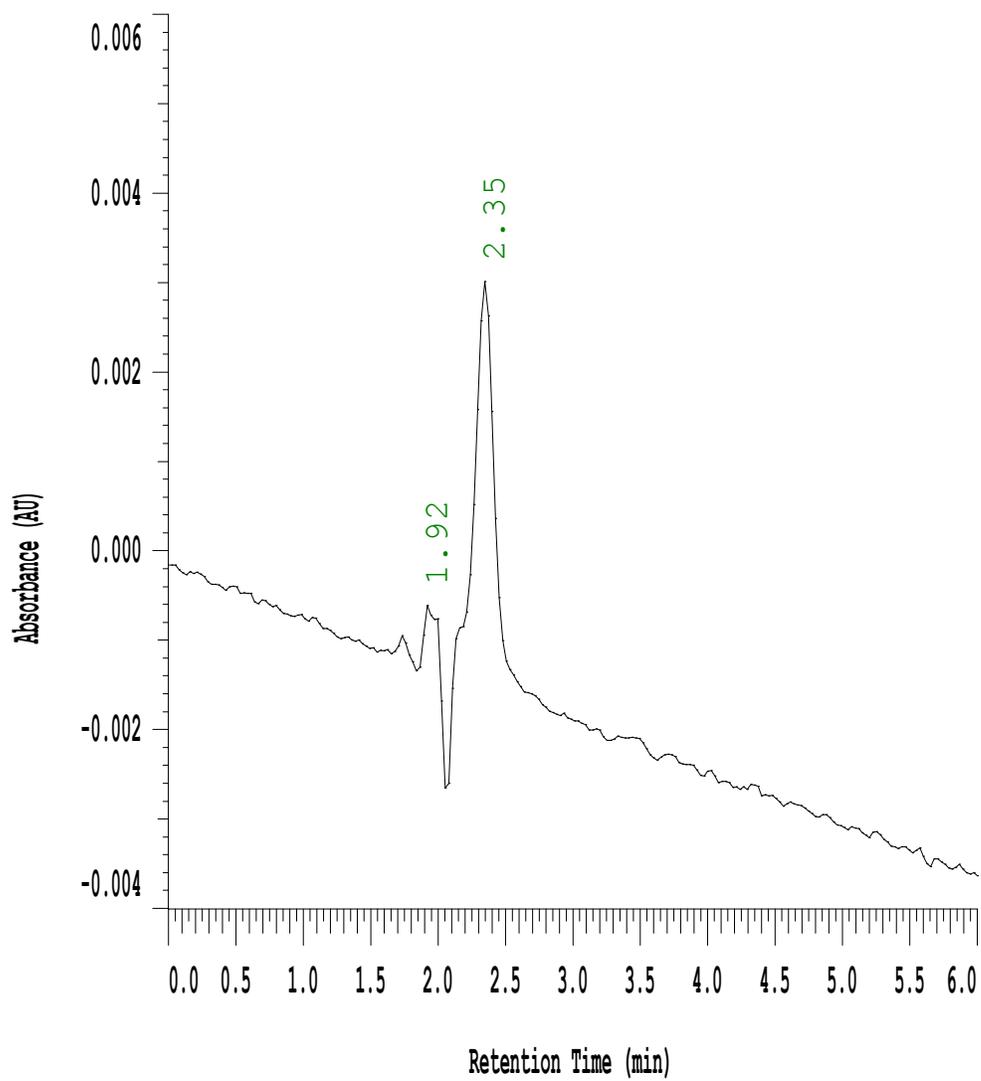


Fig 6. Chromatogram under optimized condition of unprotonated curcumin. 20 μ L of 1×10^{-4} M curcumin.

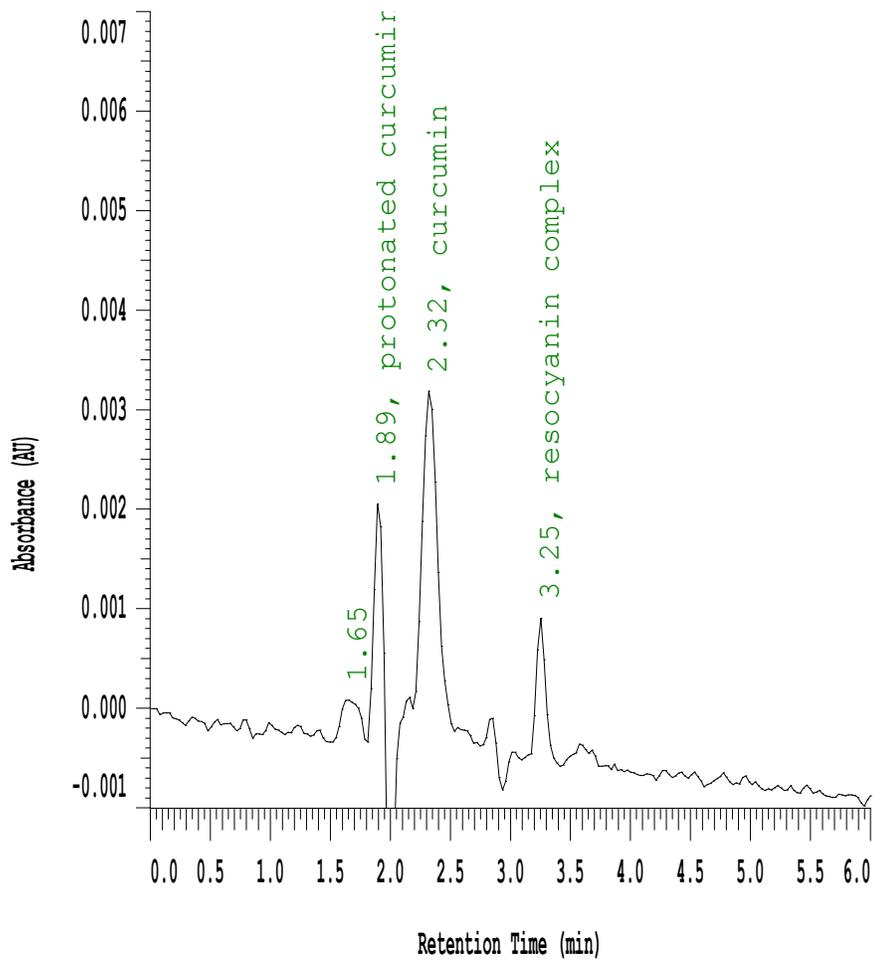


Fig 7. Chromatogram showing the different species that absorb at 550nm

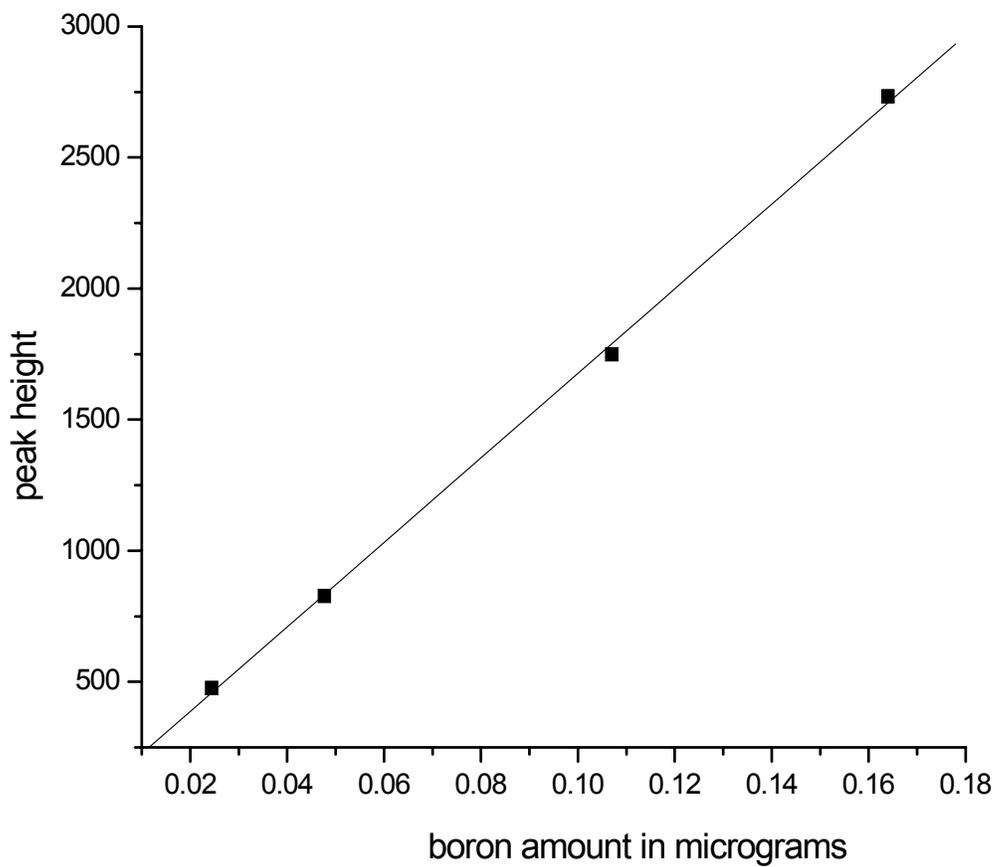


Fig 8. Calibration plot obtained for boron-curcumin complex. 20 μL of 2×10^{-8} M to 2×10^{-7} M solution was loaded into the column.