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# Determination of Bromine, Arsenic, Mercury, and Selenium in Plant by Neutron Activation Analysis

by

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# 방사화분석법에 의한 식물 중의 Br, As, Hg, Se의 정량

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## Abstract

The sensitive technique of activation analysis is well suited for this study since the elements such as As, Br, and Se in tobaccoes are expected to be high concentration.

As, Br, and Hg were determined by Bethge destruction method and subsequent neutron activation analysis. $^{77m}$Se was also by non-destruction activation analysis.

The quantities of the element determined in Korean tobaccoes are given as follows in ppm: As, 0.65 ppm. Hg, 0.74 ppm. Se, 1.18 ppm. Br, 7.1 ppm. From the date given it seems that Korean tobaccoes and foreign tobaccoes contained considerably high concentration of selenium and mercury.

## Introduction

Tobacco is one of the plant most often used in the studies on the mineral nutrition. Current interest in the production of tobacco and the physiological effects of human consumption of tobacco, make it desirable to study the inorganic microelements in tobacco products. Research up to 1964 has been admirably summarized by Tso[1]. In view of the magnitude of production tobacco and the physiological effect in human consumption and in toxic element such as arsenic,[2] selenium,[3,4,5] Po[6], in tobacco product considering the possible effect of the element in lung cancer, draw our interest.[7] $CH_3Br$ is used for preservation of cereals and in the field to combat adestructive action of plant parasote hematodes.[9,8] Arsenical compound, as insoluble arsenates, are sometimes used against fruitworms.[10] We also may mention the importance of bromine in diet studies. In this study neutron activation analysis multichannel analyzer detector has been employed. Their use in analysis of biological sample has been mineral.[12,11,13] These studies have sought to measure the elemental composition of tissue,[15,14] plants,[16] food stuffs,[17] and even cigarettes.[18,19] Many materials of known toxicity, such as Hg, As, Br and Se, lend themselves to microanalysis using neutron, activation analysis. The method, the author have determined arsenic traces using wet-ashing followed by radiochemical separation, distillation, and subrequent precipitation.[22,20,21] Bromine traces have also

been determined by distillation and subrequent separation from the distillate.[16,23] Serveral method for the determination of mercury and arsenic in biological material by means of neutron activation analysis are now available.[24,25] However, the analysis is still encumbered wtih certain problems attributed to the volatile of various mercury compounds. Thus, considerable losses of mercury during the drying of organic matter have been report.[26,27] The use of $^{77m}$Se for determining selenium by neutron activation analysis was described.[28] The work present here is confined to the abundance of trace element (Hg, Se, Br, As) known to be toxic in tobacco.

## Materials and Methods

### 1. Sampling

Cigarette and tobacco (made in Korea, U.S.A. and Japan) were sample for the determination of bromine, arsenic, mercruy, and selenium. Dry sample of the plant material is avoided to prevent losses by volatilization of the elements to be determined. [29] Tobacco sample leaves used for each brand was put into a dry oven at 70°C for 14 hr, weighed accurately and sealed in polyethylene vail. Weighed wet samples of 1 g, were sealed in a small polyethylene vial of about 1×3 cm diameter.

### 2. Irradiation

The irradiation were carried out for 15 hr on the rotary specimen rack of TRIGA MARK II at a thermal Neutron flux of $3.8\times10^{12}$n/cm$^2$sec. The sealed vials were irradiated for 5 hours as mentioned above. After irradiation, the sample were left for 3 day to let decay most of sodium-24 activity. The irradiation were carried out in the Pneumatic Rabbit system of at a thermal neutron flux of about $8.7\times10^{12}$n/cm$^2$sec. The optimal time was 0.3 min for irradiation.

### 3. Standards

The standard solution of bromine, arsenic, mercury and selenium were prepared by dissolving known weight of $NH_4Br$, $As_2O_3$, $HgCl_2$, $Na_2SeO_3$, of reagent grade. Solution was prepared with $HgCl_2$ dissolved in 1N nitric acid.

Selenium standard used on aqueous solution of sodium selenite 1 $\mu$g Se/ml for the liquid sampls. The calibration for absolute amount of selenium was carried out comparing the selenium standards with initial selenium-free samples of the plant material to which one $\mu$g selenium as sodium selenite was homogenously a mixed and irradiated.

### 4. Separation of $^{203}$Hg, $^{82}$Br, $^{76}$As and $^{77m}$Se

The method developed for selective activation analysis of mercury, arsenic and bromine are identical with respect to preparation, neutron irradiation and digestion of samples. The sample is digested in Bethge apparatus in a mixture of nitric and sulphuric acid with Hg. Mercury carrier add as described earlier elsewhere.[21,24]

Kim, also investigated a specific method for mercury analysis based on the deposition of mercury on metallic copper powder, and the mercury was collected on a copper foil by the evaporation of mercury from the

**Table I. Characteristics of the activation analysis performed for each element analysis.**

| Target nuclide | Abundance(%) | Cross-section(b) | Isotope produce | Half life | Radiation and Energy (MeV) |
|---|---|---|---|---|---|
| $^{196}$Hg | 0.146 | 880 | $^{197}$Hg | 65h | $\gamma$: 0.077 (20%) 0.19 (0.5%) |
| $^{202}$Hg | 29.80 | 3.8 | $^{203}$Hg | 46.9d | $\beta$: 0.208 $\gamma$: 0.279 (83%) |
| $^{79}$Br | 50.56 | 8.5 | $^{80}$Br | 18 min | 0.68 |
| $^{81}$Br | 49.44 | 3.5 | $^{82}$Br | 35.9 hr | 0.55 0.77 1.03 1.30 |
| $^{75}$As | 100 | 4.2 | $^{76}$As | 26.8 hr | 0.55 0.64 1.20 |
| $^{74}$Se | 0.87 | 26 | $^{75}$Se | 121 d | $\gamma$: 0.265 0.136 0.280 0.402 |
| $^{76}$Se | 9.02 | 22 | $^{77m}$Se | 17.5 sec | $\gamma$: 0.162 |
| $^{80}$Se | 49.82 | 0.5 | $^{81}$Se | 18.2 min | $\beta$: 1.38 |

[Reference (33)]

amalgamated copper powder. Determined mercury in biological materials by activation to 65-h $^{197}Hg$ and others the long-lived Hg nuclide have reported.

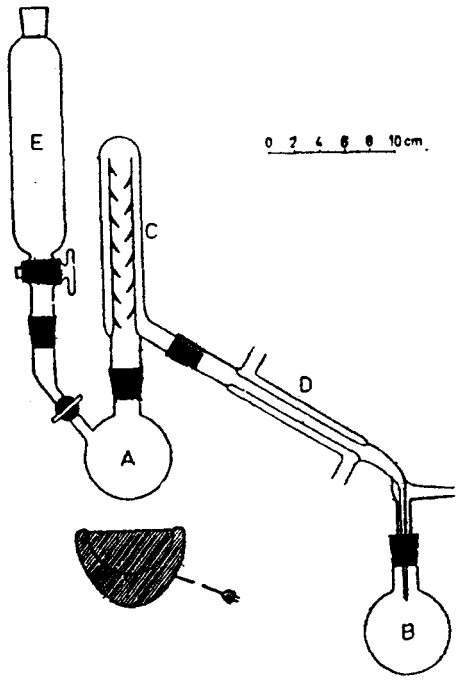


**Fig. 1. Bethge apparatus for destruction of sample**

1) Mercury seperation

One gram of collected sample was sealed in a vial. The vial was washed with nitric acid before introducing the sample. From the vials, the sample was placed to a distillation flask containing 1mg of mercury carrier. The vials was washed out with 2 ml of nitric acid. After the destruction and the cooling, the solution was taken into 100ml volume erlenmeyer flask. The distillation flask was washed out with water and added to the sample solution. The total solution was then diluted to 100 ml. Activity of solution thus became about 1 N freshly prepared (amalgamed) copper foil was suspended in the solution with polyethylene stiring. Mercury deposition was carried out during two hours. After deposition, the foil was taken out from the solution and washed with water, methanol and acetone. The foil was then sealed in a polyethylene bag for counting was performed with a sintillation counter for 5 min to 1 hour depending on the intensity of activity.

2) Bromine distillation

One mililiter of a solution containing 10 mg of inactive $As_2O_3$ is added as the As carrier. Then the sample is dried by boiling in a mixture of concentrated nitric and sulphuric acids(1 to 1 by volume). This is heated until fumes of sulphuric acid appear. The condensate is collected in 30 ml of 10 wt% NaOH solution in an ice cooled receiver flask. Under such experimental conditions bromine is evolved, while the organic matter of the sample is completely oxidized. The recovery of bromine is essentially 100%, and studies with As as tracer have shown that arsenic is not lost during bromine distillation.

Chlorine also is evolved under the same conditions as bromine, but the radioactivity of $^{38}Cl$ (half-life 37 min) has completely decayed when counting the bromine fraction.

3) Arsenic distillation

After separation of bromine, the distillation flask is cooled and 10 ml of water are added, dropwise, followed by 10 ml of concentrated HCl, 10 ml of concentrated HBr, and 2 ml of 40 wt% stannous chloride solution in concentrated HCl. The mixture is distilled for 20 min and the condensate collected in 30 ml of ice-cooled water. The recovery of arsenic as a halide is essentially 100%.

The temperature of the fumes, during the distillation, is allowed to rise to 120°C.

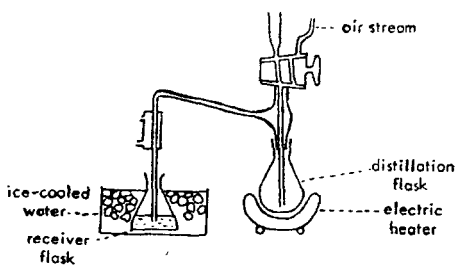


**Fig. 2. Distillation apparatus scheme**

4) Activation analysis with $^{77m}Se$.

To eliminate the critical influence of the short half-life each samples was not only irradiated a standard but also measured with selenium standard using a multichannel analyzer for detector. In this way errors due to variations in time and neutron flux are compensated as detectors used were to reduce the high-energy background above the selenium line. Finally, handling 0.3 min lifetime measuring 0.3 min cooling

and 0.3 min spectra subtraction. A few examples of $\gamma$-spectra of difference plant material are shown in Fig. 1.

### 5. Radioactivity measurement

The radioactivities of each element were measured by determining the areas of characteristic peaks which were obtained us in a (TMC 102) 100-channel pulse height analyzer. The characteristic peak of $^{76}As$ is in the energy region of 0.56 Mev. The energy regions of $^{203}Hg$, $^{82}Br$ and $^{77m}Se$ were 0.279, 0.55 and 0.162 Mev. respectively.

## Results

Results obtained from the neutron activation analysis of tobacco sample were summarized in Table 2.

Table 2 shows analytical data for tobacco sample (Hg content $\pm$0.76 ppm).

Other investigators suggested that the loss of mercury during acid digestion could be considerable.[24]

We have applied the described method to the determination of toxic element Hg, As, Br, and Se, in tobacco and several vegetable products.

Table 2 gives the results of determination of Hg, As, Br, and Se, in plant material.

The results show that harmful toxic element is of a considerable amount. Table 2 gives the results of analysis of during tobacco leaves and stems, and seven brands of commercial cigarettes. The concentration of selenium was much higher in tobacco stalks than in either tobacco leaves or cigarette filters where the concentration of selenium is much lower than in the tobacco samples.

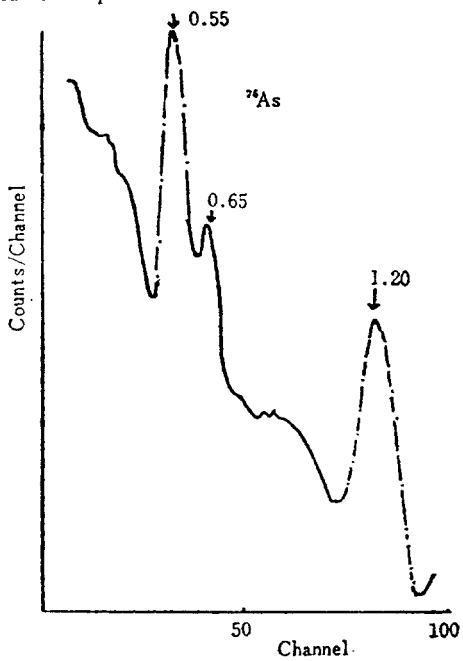


**Fig. 3. Gamma-spectrum of $^{76}As$ obtained from the irradiated sample**

**Table 2. Bromine, arsenic, mercury and selenium content in tobacco leaves**

| Sample | Concentraction determined as $\mu$g/g of dry material (ppm) | | | |
|---|---|---|---|---|
| | Br | As | Hg | Se |
| Tobacco leaves (Korea) | 132 | 0.82 | 0.96 | 1.8 |
| Tobacco stalks (Korea) | 68 | 2.9 | 0.36 | 4.8 |
| Tobacco ashes (Korea) | 5.8 | — | — | 1.3 |
| Cigar tobacco (US) | 65 | 2.2 | 0.93 | 4.3 |
| Pipe tobacco (US) | 69 | 1.68 | 0.62 | 3.4 |
| Cigarette US brand A | 36 | 0.97 | 0.21 | 0.08 |
| Cigarette US brand B | 63 | 1.70 | 0.19 | 0.06 |
| Cigarette US brand C | 47 | 1.20 | 0.31 | 0.07 |
| Cigarette (Japan) brand A | 58 | 0.62 | 0.78 | 0.04 |
| Cigarette (Japan) brand B | 67 | 0.58 | 0.92 | 0.08 |
| Cigarette (Korea) brand A | 78 | 0.76 | 0.81 | 1.21 |
| Cigarette (Korea) brand B | 65 | 0.64 | 0.92 | 1.04 |
| Cigarette (Korea) brand C | 68 | 0.65 | 0.72 | 1.35 |
| Cigarette (Korea) brand D | 59 | 0.49 | 0.67 | 0.92 |
| Cigarette (Korea) brand E | 71 | 0.68 | 0.53 | 1.12 |
| Cigarette (Korea) brand F | 84 | 0.74 | 0.95 | 1.45 |

**Table 3. Determination of Hg, As, Se, and Br in plant products by neutron activation analysis**

| Material analyzed | Hg (ppm) | As (ppm) | Se (ppm) | Br (ppm) |
|---|---|---|---|---|
| Rice | 0.115 | 0.070 | 0.180 | 15.0 |
| Barley | 0.145 | 0.050 | 0.125 | 8.8 |
| Apple | 0.105 | 0.405 | 0.102 | 1.125 |
| Bean | 0.123 | 0.326 | 0.113 | 1.36 |
| Cabbage | 0.177 | 2.38 | 0.118 | 18.50 |

## Discussion

High toxic materials from industrial and other pesticides sources are constantly being released into the environment. The hazards of these materials to the environment and their danger to mans health must be studied and understood. In order to understand such effect, it is necessary to develop comprehensive analytical method to detect these materials. Fortunately, a majority of the most dangerous chemical (ex. arsenic compounds) occur in the trace and subtrace concentrations thus in order to determine the effects of the compounds, an extremely sensitive analytical technique. The arsenic determination reported in the following have been made by means of an analytical procedure fully described in papers.[23,23] The limit of detection for arsenic is about 0.5 mg. Confidence in the values obtained by activation analysis, however, strengthened by the comparative studies organized and reported by Bowen.[30] The precision of the methods used for the determination of mercury and arsenic have been tested by repeated analysis of Bowen's kale powder.[30] In view of the number of elements of suspected toxicity present in tobacco this study is of singular interest. The techniques used in this study may also be applied to trace element analysis in a wide variety of biological matrices.[31-33] This paper presents the nature of mineral concentration in species tobacco leaf. Pesticide residue studies, the determination of Br, As, Hg and Se, toxic element is useful in other investigation areas such as biochemistry.[10] While long-standing exposure to metals such as arsenic in high concentraction is known to produce cancer in man, the role of trace element in the aetiology of cancer has not yet been studied systematically and present knowledge is far from satisfaction.

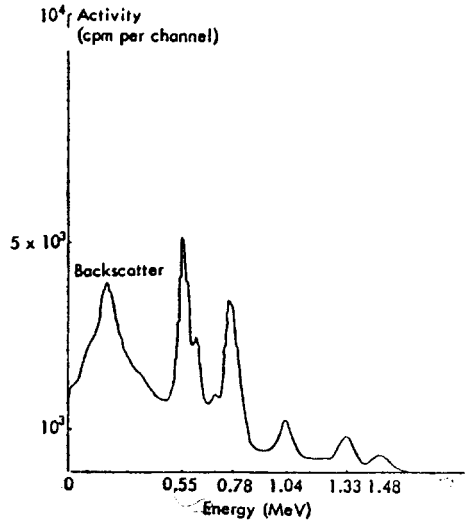


**Fig. 4. Gamma-spectrum of $^{82}$Br obtained from the irradiated sample**

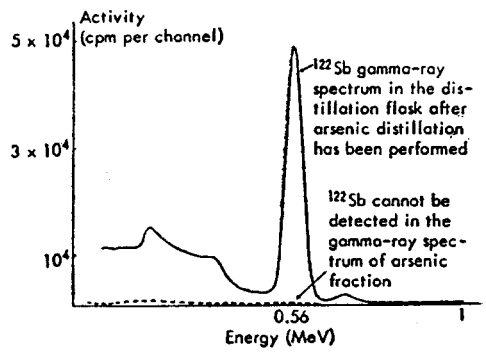


**Fig. 5. Experimental study of antimony compartment during arsenic distillation, using $^{122}$Sb as tracer**

Cancer epidemiology involves the search for substances such as these functioning as environmental factors in caution of cancer. Chronic exposure to arsenic is held to be associated with increased skin cancer levels, and poslibly with an increased incidence of internal cancer as well.[35] Chlorine also is evolved under same condition as Br, but the radioactivity of $^{38}$Cl (Half-life 37 min) has completely decayed when counting the Br fraction. The distillation is allowed to rise to 120°C under such a condition of temperature, antimony cannot be detected in the arsenic fraction, but germanium and tin are likely to distill.[33] The natural presence of Ge in plants has not been pointed out.[16]

Ge would transmate by neutron irradiation into $^{75}Ge$ (half life 82 min.), which would decay before counting the arsenic fraction. As concerns tin it has a small cross section[36] and naturally occurs at a low level in plant[16] so that it has never been detected in arsenic fraction. Finally, even if Sn were present in the arsenic fraction As and Sn could be differentiate by $\gamma$-spectrometry. Radiochemical purities of the final precipitates were determined by measuring both their X-ray spectra and decay curves. The chemical yield of these elements in the separation were about 85% for each element, and the sensitivity limit were estimated at $1\times10^{-5}\mu g$ for As, $3\times10^{-3}\mu g$ for Br, $5\times10^{-4}\mu g$ for Hg, $0.02\,\mu g$ for Se. The distillation method is thought to a convenient way to obtain selectivity and sensitivity. Detection limits depend on the plant material analyzed since an excess of $^{24}Na$ (half life 15 hr) has longer decay time and a lower accuracy. To conclude, the proposed method allows a sensitive determination of trace of Hg, As, Br, and Se.

Radiochemical separation improves the quality of the analysis compared to the instrument method, without excessive consumption of time owing to the rapid distillation process.[36] The analytical results are shown in Table I. The mercury content of Korean cigarettes was much higher than that of foreing cigarettes, the average content being 0.76 ppm and 0.23 ppm, respectively. It seems that the high mercury content of Korean cigarettes is due to the use of mercury compound pesticides on tobacco from. The amount of arsenic[24,36,37] mercury[24,38,39] normally present in the human body can now be studied, but many more data are needed for populations in different regions of the world. A parallel can be drawn between the Quebec cardiomyopathy episode and the epidemic of heart failures which occurred among beer drinkers in Manchester in 1900. The Manchester syndrome, however, was ascribed to beer contamination with arsenic as reported by Selye,[40,41] arsenic can indeed cause myocardial necrosis both in animals and in man.[42] It is for this reason that such a new topic of investigation as trace elements, which could supplement our knowledge of the aetiology and pathogenesis of cardiovascular diseases and could furnish in formation of diagostic and preventive importance, should merit our attention at the present time.


## 초 록

폐암과 담배와 밀접한 인과관계가 있음을 알게된 후, 독성원소(As, Se, Hg, Br)는 발암성 물질로서 문제화되고 있다. 특히 Se은 담배와 그 종이에도 함유되고 있음이 분석결과 나타났으며 이 무기화합물은 극히 유독하여 폐암의 원인이 되고 있지 않은가 생각된다. 그러나 이 독성원소(As, Se, Hg, Br)는 극미량이 있음으로 방사화분석으로 정량하는 것이 효과적일 줄 믿고 실험하였다.

본 실험은 한국산 및 외국제(미국산, 일본산)의 담배를 시료로 수집하여 건조한 것을 vial에 넣고 열중성자속 $3.8\times10^{12}$n/cm sec에 15시간 조사하였다. 이 시료를 Bethge 장치로 분해시켜, 수은, 비소, Br을 증류하여 TMC 100 channel pulse height analyzer로 측정하였다. Se분석은 $^{77m}Se$ 18초의 핵종을 이용하여 비파괴분석하였다. 국산담배에서는 Br 5.9~8.4 ppm, As 0.49~0.79 ppm, Hg 0.53~0.95 ppm, Se 0.92~1.45 ppm 함유하고 있으며, 이것을 외산담배와 Br, As, Hg, Se 함량을 비교한 결과를 발표한다.


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