DETERMINATION OF IODINE BY ISOTOPE DILUTION ANALYSIS IN FOOD AND BIOLOGICAL MEDIUM

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ABSTRACT
Iodine is an important trace element and is one of main constituent of thyroid hormones. In this study, a method was developed for which can be applied routine iodide analysis in environmental and biological samples. The method based on substochiometric isotope dilution analysis (IDA). Iodide concentrations have been measured in several media such as urine, drinking water, milk and commercial salts.

Key Words: Isotope Dilution Analysis, Iodine Deficiency, Iodine-131

INTRODUCTION
Iodine deficiency is a problem for almost all countries of the world with about 1600 million people (mostly in developing countries) being currently at risk of iodine deficiency disorders (IDD). World Health Organization (WHO) recommendation of daily iodine intake is in 50-200 µg [3]. The term endemic goiter is used when more than 10% of population or 5% of an adolescent group have goiter. According to WHO documentation, the 24 hour urinary excretion is less than 50 to 99 µg/L in endemic areas [3]. The negative effects of iodine deficiencies on growth and development are called iodine deficiency disorders. Although all age groups can be effected from iodine deficiency, the most risky groups are pregnant, fetus, new born and infant. The most common consequence of iodine deficiency is goiter on all age groups. Goiter rate is accepted as reliable indicator of iodine deficiency in a population. Besides goiter low school success and low IQ level are the other important consequences of iodine deficiency. Although WHO has aimed that have iodine deficiency disorders disappeared up to 2000, iodine deficiency disorders have still been seen in our country as many other countries in the world, and effects negatively development and economies [4-6, 14, 9]. Because most iodide excreted in the urine, urinary iodide excretion is currently the most convenient laboratory marker of iodine deficiency [7,8,18].

This report contains a review of some studies about iodide concentrations measured by IDA in several media such as urine, drinking water, milk and commercial salts [10, 11, 14].

MATERIALS AND METHODS
Materials: Gelman paper electrophoresis equipment was used for electrophoresis procedures. Tennelec PCA II 8196 Channel Analyzer equipped with a 3x3 NaI(Tl) well type scintillation detector was set for counting procedures.
Reagents: All chemicals were purchased from Merck. Na$^{131}$I was supplied by Department of Nuclear Medicine. All solutions were prepared in doubly distilled water. Potassium Iodide Standards: 40000 µg/L stock KI solution was prepared by dissolving 40 mg of KI in 1000 ml doubled distilled water. From this working standard, further dilutions were made to give an effective concentration of KI in the range of 5-5000 µg/L. These solution were stored in dark. 0.1 N Na$_2$SO$_3$ and 2.10$^{-6}$ N AgNO$_3$ solutions were prepared.

Collection of samples: Urine samples were collected randomly by 318 healthy children by Pediatric Endocrinology group of Ege University. Drinking water samples which were collected from 28 settled place were belong to these children. They were tap water, well water, deionized water or bottled commercial water. Milk samples were collected from Izmir and environmental municipalities and these were winter season products. Salt samples were iodized or noniodized commercial salts consumed in Turkey.

Sample Preparing Procedures: Water and urine samples were used without any application procedure. Salt Samples: One gram salt sample was dissolved in 3 ml distilled water. Then it was used as the same procedure with the urine and water samples. Milk Samples: 4 ml 4 N NaOH was added to 100 ml milk sample, then it was evaporated on a hot plate. It was made ash at 600 °C in an ash-oven for 8 hours. 5 ml concentrated HCl was added then made up to 100 ml with distilled water.

Analysis Procedure:

Five µL of sample which will be analyzed were put in each of a series of tubes. Equal volumes with increasing iodide concentrations and equal volumes of identical $^{131}$I solutions were added to dissolved samples. Less than equivalent amounts AgNO$_3$ were added and they were rested for 15-20 minutes. Five µL of Na$_2$SO$_3$ to prevent oxidation of iodide and 5 µL of dioxan were added, consecutively. Five microlitres of each test tube was transferred to cellulose acetate electrophoresis strips premoistened by buffer solution. Electrophoresis was performed with a Gelman electrophoresis chamber. Buffer solution was a mixture of n-butanol/water/acetic acid (4/2/1). Migration time and applied voltage were two hours and 300 volts respectively. While excess $^{131}$I migrates due to electrophoresis on paper, Ag$^{131}$I precipitate remains stationary and this fraction of paper is counted with a NaI(Tl) scintillation detector of multichannel analyzer using the gamma peak of 364 keV of $^{131}$I. These count rates were plotted versus iodide concentrations by using a computer Curve Fit program. A linear decreasing plot was obtained and used as a calibration curve. Three parallel experiments were performed with each sample.

RESULTS AND DISCUSSION

The precision of the method was evaluated using standard solutions within the range of 7-7500 µg/L previously described [10-11]. Each sample was analyzed at least three times. It was observed that the entire range our values are in agreement within a maximum error of ± 10.15. Thus the procedure yields accurate results at the microgram level. Minimum detection limit was determined as 1 µg/L. Relative standard derivations were not higher than 14%.
Figure-1 shows the frequency distributions of drinking water concentrations [10,14]. Iodide concentration's ranges are within 1.8-100.45 μg/L in analyzed drinking water concentrations. Mean value is 44.13±17.33 μg/L, median is 58.08 μg/L. Figure-2 shows the frequency distribution of urinary iodide in children [14]. The median of the distribution is 37.71 μg/L, maximum urinary concentration is 142.22 μg/L, minimum is 0.48 μg/L and mean is 40.30 ± 24.05 μg/L. When estimates of iodine intakes derived from urinary iodide excretion values were compared with a graded scheme of severity for endemies of iodine deficiency disorders, 22.95% of children suffer severe iodine deficiency, 46.22% moderate, 30.18% mild. These results show that west part of Aegean region has moderate iodine deficiency. On the other hand, pediatric endocrinology group of Ege University has also been reported that goiter prevalence is 43% of school children of some part of Ege Region [13].

Frequency of iodide concentrations of Turkey's salts is given at figure-3. According to these results, salts consumed in Turkey contain between 9-58 μg/g iodide and the mean iodide concentration is 27 μg/g. Brand 1 and brand 2 (iodized) are products of well-known companies who sell their products with wide publicity assuring that the salt is iodized. Both the manufacturers had assigned a minimum iodide concentration of 50 μg/g. The noniodized product of brand 1 also contains approximately 30 μg/g iodide. Brands 3, 5, 6 and 9 contain less than 30 mg/kg while iodide concentrations of brands 4, 7 and 8 are higher than 30 μg/g. Mineral origin salt also contains iodide (29 μg/g).

Iodide concentration of salts vary greatly in other countries. In Latin America, the concentration of iodide is between 30-100 μg/g, in Europe between 10-20 μg/g [11], in India 30 μg/g [9], in some of the African countries, like Gobe, Moritan, 0.1 μg/g [12]. Turkey’s salts have iodide concentrations similar to Indian salts, but much higher than Africa-Gobe salts.

Daily salt consumption largely depends on the individual food habits in Turkey. Iodination of salt in Turkey is not considered mandatory up to June 1998 and subvention of iodination does not exist. Advised iodide concentration in salt is 50-70 μg/g in Turkey. As pointed out in some reports, mandatory prophylaxis is usually more effective than a voluntary one, but there are also examples of good results on a voluntary basis [1,2,4]. For this reason, iodine deficiency may not be prevented by iodinated salts and nutritional habits of population may be more important because of the 90 % of iodine ingested by solid food.

Figure-4 shows the frequency distributions of iodide concentrations of milk samples. Iodide concentration's ranges are within 58.78 ± 7.40 μg/100g and 51.85 ± 7.32 μg/100g. Mean value is 55.65 ± 2.86 μg/100g. These values are agree with WHO report deal with the iodide concentrations values in milk. Mean iodide concentration is given 56 μg/100g in milk by WHO [3]. According to these results animals which have been used consumption of milk have no iodide deficiency at this region. The reason of why these animals have been fed by EDDI (ethylene di amine di hydro iodide) added grains to get good produce. However population in
Turkey are not a good consumer for milk. On the other hand these samples were the winter production. Levander and Whanger reported that iodide concentration in milk is very changeable season to season and iodide concentration may change up to eight times [16], since the animals have been fed by dry grains to which have been added some supplements such as EDDI for winter season. However they have been fed by fresh grains for summer season.

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Figure-3: Frequency distribution of iodide concentrations in Turkey's salt (μg/g).

Figure-4: Frequency distributions of iodide concentrations in milk (μg/100g).