DETERMINATION OF TRACES OF LEAD IN TINNED APPLE PUREE BY LIQUID CHROMATOGRAPHY WITHOUT SAMPLE MINERALIZATION

František VLÁČIL and Věra HAMPLOVÁ

Department of Analytical Chemistry,
Prague Institute of Chemical Technology, 166 28 Prague 6

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A method has been worked out for the direct determination of lead in apple pulp without previous sample mineralization, based on the HPLC analysis of metal diethyldithiocarbamates isolated from the apple pulp by extraction with chloroform after the addition of reagent and masking iron with KCN. The amount of lead isolated by the direct extraction is the same as that obtained by mineralization. The method suggested gives results identical with those of the standard procedure of photometric determination with dithizone after mineralization; the precision and limit of determination (0·2 ppm Pb) are also approximately the same for the two methods. The recovery in the new method is 89%, as compared with the 94% for the standard method.

The monitoring of lead content of tinned apple puree is of importance since apple fruit can be contaminated by lead present in airborne matter and the product can be additionally contaminated from the tin soldering of the metal container. Of particular concern is the contamination of children's apple puree because according to WHO, the maximum allowed amount of lead absorbed by a human within a week is 50 μg per kg weight for adults and even less for children.

Traces of heavy metals in food are usually determined by AAS after sample mineralization, although methods of direct determination without mineralization are actually preferable with regard to the loss of lead and also the possible contamination of sample during the mineralization[1]. Flameless AAS (refs[2,3]), direct anodic stripping voltammetry[4,5] or X-ray fluorescence[6] have been suggested for the direct determination of lead in tinned fruit.

Since HPLC is being increasingly used in food control laboratories, we examined the possibility of determining lead by the liquid chromatography of its chelate using a reversed-phase system. Of chelates so far studied, diethyldithiocarbamate gave the best results for the HPLC of heavy metals[7,8]. Lead is likely to be present in apple pulp in the form of covalent compounds (*e.g.*, with anthocyanines, citric acid, pectin, *etc.*), but these complexes probably will not be very stable. Pectin, for instance, was found to bind lead in alkaline media only[9]; the stability constant (β) of lead chelate with D-galacturonic acid (the monomeric unit of pectin) is[9] $\log \beta = 2{\cdot}51$ and with

citric acid, $\log \beta = 12{\cdot}3$ (ref.[10]) at pH $\approx 7{\cdot}5$, whereas the so-called two-phase stability constant, $\varkappa_{ex} = \beta_n K_D$, where K_D is the extraction distribution constant of chelate, is $\log \varkappa_{ex} = 20{\cdot}9$ for lead diethyldithiocarbamate and the water–tetrachloromethane system[11]. This suggests that it may be possible to extract diethyldithiocarbamates of some metals from apple pulp without its prior mineralization and separate lead from this chelate mixture and determine it by high performance liquid chromatography. It was the purpose of this work to verify this assumption and to compare the results so obtained with those of the standard method of determination of lead in food[12].

EXPERIMENTAL

Chemicals and Solutions

Nitric acid was of *s.p.* grade (Lachema, Brno), the other chemicals were of *p.a.* grade. Deionized water was additionally distilled in an all-glass apparatus, and filtered through a G 4 frit for chromatographic purposes. Methanol was rectified on a column; its transmittance at 250 nm in a 1 cm cell was better than 95%. Tetrahydrofuran was dried over potassium hydroxide and rectified with sodium; its transmittance at 260 nm and above 280 nm, in a 1 cm cell, was 70% and better than 90%, respectively. Chloroform was purified according to ref.[13]. The mobile phase for chromatography, methanol–water mixture ($\varphi(H_2O) = 0{\cdot}25$), was degassed by boiling under reflux for 5 min and rapid cooling to room temperature. Aqueous solution of dithizone was prepared daily by shaking 25 ml of 0·1% solution of reagent in chloroform with 40 ml of 0·5M-NH_3. The aqueous phase was filtered, and the solution was stored in a refrigerator even during the work. Solution of KCN, also prepared daily, was purified by extraction with 0·1% dithizone solution in chloroform followed by four-fold extraction of the aqueous phase with chloroform. The stock solution of lead was prepared from lead nitrate and its concentration was determined by chelatometric titration. Working solutions containing lead in a concentration of 1 mg l^{-1} were prepared daily by dilution of the stock solution and acidification with nitric acid to pH 2 to reduce the extent of lead adsorption on the walls of glass vessels[14,15].

Apparatus

A Varian 4 000 liquid chromatograph (Varian, Palo Alto) equipped with a septum injector (pressure max. 7 MPa) and with a column 250 mm × 6 mm i.d. packed with Separon SI C 1 (silica gel with chemically bonded methyl groups), $d_p = 10$ μm (Laboratorní přístroje, Prague) was used in conjunction with a photometric detector of Varian working at 280 nm, with a cell volume of 8 μl. The sample absorbances for the photometric analysis were measured on a VSU 2-P spectrophotometer (Carl Zeiss, Jena). The extracts were preconcentrated in a concentrator after Kuderna and Danish[16] and in a rotary vacuum evaporator.

Vessel Pretreatment for Trace Analysis

Polyethylene vessels were cleaned[17] by allowing them to stand with HCl (1 : 1) for a week, rinsing them thoroughly with distilled water, and allowing them to stand with HNO_3 (1 : 1) for another week. After thorough washing with distilled water, the vessels were filled with specially purified distilled water and stored in a closed glass vessel; the water in them was replaced every

third day. Vessels from borosilicate glass were washed successively with a detergent solution, distilled water, HNO_3 (1 : 1), distilled water, and specially purified water, and dried and stored on a dustfree place. Porcelain ashing dishes were allowed to stand with HCl (1 : 1) for 24 h, rinsed with distilled water and boiled in HNO_3 (1 : 1). After rinsing with distilled water and specially purified water, the dishes were annealed in a furnace at 450 ± 20°C.

Sample Pretreatment

Samples of tinned children's apple puree (Deva, Nové Město nad Metují) were analyzed. After opening the tins, all pieces of metal were carefully removed from the food. The tin contents were homogenized and transferred to a polyethylene bottle and stored in a refrigerator. For the determination of the dry substance fraction, the sample was mixed with the same amount (by weight) of predried quartz sand and dried at 105° to constant weight.

Sample Mineralization

20—40 g of sample with 10 ml of 10% $Mg(NO_3)_2 . 6 H_2O$ solution was evaporated[12] on an ashing dish to dryness, the residue was charred under a heating lamp and ashed at 450 ± 20°C. The cold, light-colour ashes were wetted with water, 10 ml of 10% HCl was added, and the whole was heated on a water bath for 15 min. The insoluble residue was filtered out and the filter paper wetted with magnesium nitrate solution was dried again in the ashing dish, charred, and the residue was ignited at about 450 °C. The ashes were digested with 5 ml of 10% HCl and the solution was filtered again. The combined filtrates were diluted to 100 ml.

Analysis

Procedure A (ref.[12]). To a 25 ml aliquot of the solution from the mineralization were added 5 ml of 5M-HCl and 2 ml of 25% ammonium citrate solution, and the mixture was alkalinized with ammonia using thymol blue as the indicator (colour change from yellow to blue-green). To this system were successively added 1 ml of 10% KCN, 1 ml of 10% $NH_2OH.HCl$, and 10 ml of chloroform, the whole was diluted with specially purified water to 50 ml of the aqueous phase, and 0·5 ml of aqueous solution of dithizone was added. After the extraction, the chloroform layer was transferred into another separatory funnel, and the aqueous phase, to which 0·3 ml of aqueous solution of dithizone was added, was extracted with 5 ml of chloroform. The combined extracts were shaken with 10 ml of dilute HNO_3 ($\varrho = 6{\cdot}5\ g\ l^{-1}$), the chloroform layer was drawn off, and to the aqueous phase 30 ml of solution obtained by mixing 68 ml of concentrated ammonia, 136 ml of water, 6 ml of 10% KCN, and 0·3 g of Na_2SO_3 was added. 10 ml of chloroform and 0·8 ml of aqueous dithizone solution were pipetted to the mixture and the whole was extracted. The chloroform layer was then filtered and its absorbance was measured in 3 cm cells at 520 nm against water.

For obtaining the calibration dependence, the aliquot was replaced by standard solution of lead nitrate containing 0—10 μg Pb and 10 ml of 5M-HCl; further was proceeded as above. The regression straight line equation was calculated from the absorbances measured. The blank experiment was performed in the same manner, including the mineralization.

Procedure B. An aliquot of the sample after mineralization was neutralized with dilute ammonia (1 : 1) to pH 8, 20 ml of citrate buffer of pH 7·6, 1 ml of 10% KCN solution, and 2 ml of 0·2% sodium diethyldithiocarbamate solution were added, and the whole was extracted with 20 ml of chloroform. The organic phase was drawn through a filter paper into a concentrator after Kuderna and Danish and the aqueous phase was extracted with 2 × 10 ml of chloroform. The volume of the combined extracts was reduced to 3—5 ml by evaporation and the remaining chloroform was

removed at room temperature in a rotary vacuum evaporator. The residue was dissolved in a precisely measured volume (300–500 μl) of tetrahydrofuran. Five to ten μl of this solution was injected into the column; aqueous methanol was used as eluent at F_m 0·50–0·70 ml min^{-1}. The lead content was evaluated based on the peak height. A linear calibration plot was obtained over the region of 0–350 ng Pb.

The blank experiment was performed with all the chemicals used in the procedure.

Procedure C. A 40–60 g sample was diluted with 100 ml of specially purified water, 20 ml of citrate buffer of pH 7·6 and 1 ml of 10% KCN solution were added, and the suspension was extracted with 30 ml of chloroform. The organic phase was drawn off, 2 ml of 2% sodium diethyldithiocarbamate solution was added to the aqueous phase, and the latter was extracted with 20 ml of chloroform. The two phases were separated by centrifugation, and the organic phase was filtered through a filter paper into a concentrator after Kuderna and Danish. The aqueous phase was extracted with 2 × 10 ml of chloroform. Further proceeded as in procedure *B*.

RESULTS AND DISCUSSION

Superior to Separon SI C 18 (ref.[18]), Separon SI C 1 was used as the sorbent for the HPLC of the metal diethyldithiocarbamates. The mobile phase composition was optimized according to ref.[19]. The iron content of apple pulp exceeds extremely its lead content. At the pH used during the extraction, iron passes into the extract and its chromatographic peak overlaps the peak of lead. Since sulphosalicylic acid was insufficient to prevent extraction of the iron chelate, iron was masked with cyanide. The excess of sodium diethyldithiocarbamate used in the extraction must be low enough to prevent partial overlap of the peaks of lead diethyldithiocarbamate (k = 3·6) and the free carbamic acid (k = 2·8).

The assumption that sodium diethyldithiocarbamate transforms all lead compounds present in apple pulp into the extractable chelate was verified by comparing the results from two procedures (*B* and *C*) which differed in that in the one case, the sample was mineralized and heavy metals were extracted as the diethyldithiocarbamates from the ashes, whereas in the other case, the extractable chelates were obtained by direct action of the reagent on the sample. Four samples of children's apple puree from different production batches were analyzed by the two methods. The results are given in Table I along with those obtained by the standard procedure (*A*).

The agreement of results obtained by methods B and C was tested by Student's criterion[20]. The corresponding values were t = 3·400 and critical t_α = 4·303 for the α = 0·05 significance level and number of degrees of freedom ν = 2 for samples No 1–3 (without centrifugation), and t = 0·244, t_α = 2·447 (for α = 0·05 and ν = 6) for the procedure including centrifugation. Thus, the difference between the average lead contents of the samples obtained by methods *B* and *C* is not statistically significant, and the agreement of the results is better still if centrifugation is applied after the extraction. So it can be concluded that the amount of lead isolated as the diethyldithiocarbamate by direct extraction is the same as that isolated from the apple pulp on its mineralization.

Since no reference standard of apple pulp of known lead content was available, the accuracy of the lead determination by procedure *C* was evaluated in two ways *viz.*, by comparing its results with those of the standard procedure (*A*), and by applying the standard addition approach. The results are also given in Table I.

The differences between the results of procedures *A* and *C* are statistically insignificant, as revealed by the values of $t = 2{\cdot}375$ for samples No 1–3 ($t_\alpha = 4{\cdot}303$ for $\alpha = 0{\cdot}05$, $\nu = 2$) and $t = 0{\cdot}194$ and $0{\cdot}0412$ for samples No 4 and 5, respectively, for which $t_\alpha = 2{\cdot}447$ ($\alpha = 0{\cdot}05$, $\nu = 6$). Thus, the accuracy of determination of lead by HPLC after its direct extraction is the same as that of the determination by the standard method after sample mineralization. From the standard addition experiment it can be deduced that the recovery in the standard method is 94%, in the HPLC method, 89%.

The precision of procedures *A* and *C* was compared in terms of the standard deviations, which were $0{\cdot}0358$ for the standard method and $0{\cdot}0454$ for HPLC without mineralization. According to the *F*-test, the difference between the standard deviations is statistically insignificant, hence, the chromatographic method without mineralization is as precise as the standard method.

The limits of determination, calculated as ten times the standard deviations of blank experiments[21], were 39 and 43 μg Pb/kg initial sample for the standard method and the chromatographic method, respectively.

The lead content of the samples analyzed was 1–3 mg per kg of dry sample matter, the dry matter content of the pulp being 20–22%.

TABLE I

Parameters of the determination of lead by the standard method (*A*), HPLC after mineralization (*B*), and HPLC after direct extraction (*C*): mass fraction found (w), number of replicate determinations (n) and range (R)

Sample No	*A*			*B*			*C*		
	$10^7 w$	n	$10^8 R$	$10^7 w$	n	$10^8 R$	$10^7 w$	n	$10^8 R$
1	3·19	4	8·2	3·36	3	9·1	2·92[a]	2	4·6
2	4·85	4	9·7	4·31	3	8·4	3·17[a]	2	0·2
3	3·92	3	6·7	3·41	4	13·1	2·97[a]	2	8·0
4	3·50	4	4·6	3·61	4	6·7	3·66[b]	4	10·7
5[c]	6·14	4	10·7	—	—	—	6·15[b]	4	12·6

[a] Without centrifugation after extraction; [b] extraction followed by centrifugation; [c] sample No 4 with a standard addition corresponding to $w = 2{\cdot}81 \cdot 10^{-7}$.

It can be concluded that the novel method based on HPLC following the direct extraction of sample is as accurate and precise as the standard method, and the former is even superior to the latter as far as the simplicity and rapidity of analysis is concerned. The limit of determination by the two methods are also the same. The method suggested can also be applied, without modification, to the determination of lead in apple fruit, apple juices and other apple products.

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