Analysis of Cobalt in Plasma by Electrothermal Atomic Absorption Spectrometry

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Analyse von Kobalt in Plasma mit Hilfe der elektrothermischen AAS

Zusammenfassung. Bei dem beschriebenen Verfahren wird die Probe mit Hilfe von Salpetersäure/Perchlorsäure aufgeschlossen. Als Komplexierungs-Reagens wird Ammoniumpyrrolidindithiocarbamat zugefügt und nach pH-Einstellung auf 9 ± 0,1 wird Co mit Methylisobutylketon extrahiert.

Mit Hilfe von Co-60 wurde festgestellt, daß die durch Aufschluß und Extraktion verursachten Verluste weniger als 3% betragen. Eine mögliche Verunreinigung durch die benutzten Stahlnadeln wurde untersucht und diskutiert. Der Variationskoeffizient beträgt 9,5% bei 0,2 µg Co/l. Die Bestimmungsgrenze nach diesem Verfahren liegt bei 0,06 µg Co/l. Die Kobaltkonzentration im Plasma von 32 Kontrollpersonen wurde zu 0,15 ± 0,07 µg Co/l bestimmt. Für 10 Raffineriearbeiter, die täglich $CoCl_2$-Aerosolen ausgesetzt waren, wurde ein Durchschnitt von 9,7 ± 7,1 µg Co/l gefunden.

Summary. A method for the determination of traces of cobalt in plasma is described. The sample is digested in a test tube using a mixture of nitric and perchloric acid. Ammoniumpyrrolidine dithiocarbamate is added as chelating agent, and after pH-adjustment to pH 9 ± 0.1, cobalt is extracted into methyl-isobutyl ketone.

Recovery studies using Co-60 showed that losses of cobalt during the procedure, including digestion and extraction are less than 3%. Possible contamination from the steel needle used is tested and discussed. The coefficient of variation for the method is 9.5% at the 0.20 µg/l level. A realistic determination limit following this procedure is estimated to be 0.06 µg/l. Using this method, the cobalt concentration in plasma from 32 control persons was determined to 0.15 ± 0.07 µg/l. For 10 refinery workers daily exposed to aerosols of $CoCl_2$ the determined average was 9.7 ± 7.1 µg/l.

Introduction

Cobalt is an essential element as part of the cyanocobalamine complex B_{12}. This vitamin is essential to deoxyribonucleic acid synthesis and propionate metabolism.

That cobalt stimulates erythropoiesis in rats has been recognized for more than 50 years. Waltner and Waltner [1] in 1929 produced erythropoiesis in rats by oral administration of various cobalt compounds and by parenteral injection of $CoCl_2$ and $Co(No_3)_2$. Also Orten et al. [2] produced erythrocytosis in rats by oral administration of $CoCl_2$ and $CoSO_4$. Therapeutic efficacy of cobalt compounds for stimulation of erythropoiesis in anemic patients was first reported by Kato et al. [3].

Several papers have been published on the determination of cobalt in whole blood, plasma and serum.

Versieck et al. [4] analyzed cobalt in serum from 14 healthy individuals by a neutron activation method. They found the normal cobalt value to be 0.160 ± 0.083 µg/l.

Barfoot and Pritchard [5] examined both blood and serum for cobalt by electrothermal atomic absorption. After wet digestion of the sample, cobalt was extracted from the aqueous phase with 1-nitroso-2-naphthol in chloroform. The mean concentration reported was from 0.20 to 0.28 µg/100 ml for blood and from 0.12 to 0.20 µg/100 ml for serum.

Delves et al. [6] analyzed cobalt in blood by flame atomic absorption. The sample was digested in a mixture of nitric acid, perchloric acid and sulphuric acid. Cobalt was 100% extracted with APDC in MIBK at pH 2.8 – 3.3. The reported result for cobalt was 0.4 ± 0.6 µg/100 ml.

Massen et al. [7] described a direct flameless atomic absorption method for the determination of cobalt in blood plasma. The reported results range from 0.5 – 1.6 ppm.

Other investigations have reported results from 0.02 to 370 µg/l [8 – 24] as normal values for cobalt in whole blood serum and plasma. It seems clear that these widely different results cannot only arise from variation in natural blood cobalt levels of the subjects, but must also be due to analytical and methodological problems.

Here we describe and evaluate a method for the determination of cobalt in plasma for normal subjects and for exposed persons as well. The method is simple and can routinely be used in industrial laboratories not specialised in this type of analysis.

Experimental

Sampling and Sample Preparation

Whole blood samples (15 ml each) were obtained by vein puncture and collected in heparinized vacutainer tubes (Becton-Dickinson, Rutherford, NM, USA). Immediately after collection the samples were centrifuged. The plasma was withdrawn, and transferred into the digestion tube.

The control samples were collected from people living in the Kristiansand area, but not in the neighbourhood of

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Falconbridge. Samples from exposed persons were collected in the morning, and in the afternoon. This was done on Monday, Wednesday and Friday during the same week.

Apparatus

Measurements were made with a Model 603 atomic absorption spectrophotometer equipped with a graphite furnace (Model HGA-76; Auto-Sampling System, AS-I), and Model 56 recorder, all from Perkin-Elmer Corp., Norwalk, CT, USA. Adjustments were carried out as described in the suppliers manual. The instrumental conditions were:

Cobalt lamp current, 30 mA; Cobalt lamp wavelength. 241 nm; Entrance slit, position 3; Recorder range, 2 mV; Mode of operation: Peak height.

Graphite furnace program

	Temperature control setting	Time (s)	Temperature (°C)
Evaporation	1	20	140
Decomposition	2	10	420
Ashing	3	10	1,050
Atomizing	4	15	2,600

The last temperature step was done with a "miniflow" of argon gas for 10 s and then full flow for 5 s.

Reagents

All reagents were "super-pure" grade (E. Merck, Darmstadt, FRG), except as indicated. Doubly distilled water; Ammonium-pyrrolidine dithiocarbamate (BDH Chemicals Ltd., Poole, England), purified by fourfold extraction with methyl isobutyl ketone (0.12 mol/l); Cobalt 99,9% purity; m-Cresol purple indicator; Ammonia, 13.35 mol/l; Nitric acid, 14.44 mol/l; Sulphuric acid, 18.01 mol/l; Perchloric acid, 11.64 mol/l; Methylisobutyl ketone, p.a. reagent grade; Nitric acid/perchloric acid mixture (3:1 by volume).

Procedure, Control Sample

After centrifugation of the whole blood, 6 ml plasma is transferred to a 25-ml glass-stoppered test tube, 4 ml of the $HNO_3/HClO_4$ mixture is added. Place the test tube in a temperature-programmable aluminium heating block and raise the temperature to 120° C for 30 min, then increase it to 170° C for another 30 min. Thereafter increase the temperature to 210° C for complete decomposition.

After cooling, add 0.5 ml of HCl, 6 mol/l and 5 ml of dionized water, heat to dissolve, add one drop of m-cresol purple and 0.3 ml APDC. Adjust pH to 9 ± 0.1 by dropwise addition of dilute ammonia solution. Add 0.5 ml MIBK, and shake the solution for 1 min. Separate the phases by centrifugation for 2 min. Transfer 0.3 ml of the organic phase into a 3-ml Pyrex test tube, place in the AS-1 rack, and start the automatic HGA sequence program.

100 µl organic phase was aspirated into the graphite furnace in portions of 20 µl. For the first four injections the sequence program was terminated after the ashing step. After the fifth injection the complete sequence program was run.

Table 1. Results for 10 different plasma analyses (µg Co/l) from the same plasma pool

1-a	0.190
1-b	0.230
1-c	0.185
1-d	0.205
1-e	0.220
1-f	0.205
1-g	0.250
1-h	0.205
1-i	0.225
1-j	0.195
Average	0.211 ± 0.020

Procedure, Pathological Sample

This procedure deviates from the method described for control samples only in the sample volume and the amounts of reagents used. The increased cobalt concentration makes 4 ml plasma a suitable sample volume to use. Consequently, the amount of acid used for digestion can be reduced to 3 ml. 1 ml methyl-isobutyl ketone was used for extraction. Finally, 20 µl organic phase were aspirated into the graphite furnace.

Preparation of Calibration Curve

Calibration curve was established by use of cobalt spiked plasma samples. For comparison a standard curve for cobalt extracted from deionized water was also prepared. A stock solution was prepared by dissolving 0.1 g of the electrolytical pure cobalt in nitric acid and diluting to 1 l, giving a solution of 100 mg/l. Working standards were made by adding 0.5 ml of 25, 50 and 100 µg/l solutions, respectively to 6 ml of plasma or distilled water to give a final concentration in 1 ml of methyl-isobutyl ketone extract of 12.5, 25 and 50 µg/l. Calibration curves have been found to be linear up to 50 µg Co/l.

Results

We investigated the detection limit and the reproducibility of the method. 65 ml plasma were digested in a 200-ml beaker. Thereafter, 10 6-ml portions were transferred to test tubes and analyzed.

The mean cobalt concentration for the 10 samples was 0.21 µg/l, and the standard deviation was ± 0.020 µg/l. Table 1 shows the individual results with mean and standard deviation. The recorder deflections for the same are shown in Fig. 1.

In this laboratory heparinized vacutainer tubes equipped with steel needles are routinely used for sample collection. By analyzing 5 steel needles, an average of 0.2% cobalt was found. For that reason the needles could not be overlooked as a potential source of contamination. Therefore, 10 samples of blood were collected from the same person. Five samples were collected with a "vacutainer" stainless steel canulla, the other five with a "venflon" canulla with a teflon catheter. The samples were centrifuged and the plasma analyzed for cobalt. The results are shown in Table 2.

The results in Table 3 show the cobalt concentration, the mean and standard deviation, in plasma from 32 healthy

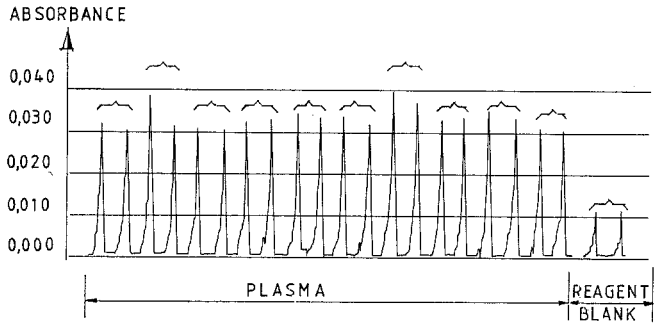

Fig. 1. Recorder deflections for 10 different plasma analyses from the same pool

Table 2. Cobalt concentration in 5 plasma samples collected by "vacutainer" compared to 5 samples collected by "venflon" cannula with teflon catheter

	"Vacutainer" stainless steel cannulla (µg Co/l)	"Venflon" cannulla teflon catheter (µg Co/l)
Sample 1	0.24	0.12
Sample 2	0.12	0.30
Sample 3	0.12	0.12
Sample 4	0.24	0.24
Sample 5	0.12	0.12
Average	0.17 ± 0.07	0.18 ± 0.09

Table 3. Cobalt concentration (µg/l) in plasma from 32 healthy individuals not exposed to cobalt

1	0.186	17	0.116
2	0.318	18	0.116
3	0.239	19	0.107
4	0.186	20	0.107
5	0.174	21	<0.130
6	0.203	22	0.092
7	0.318	23	0.162
8	0.231	24	0.06
9	0.174	25	0.078
10	0.231	26	0.233
11	<0.06	27	0.162
12	0.145	28	0.254
13	<0.06	29	0.134
14	0.116	30	<0.06
15	0.087	31	<0.06
16	0.087	32	0.120

$\bar{x} = 0.150 \pm 0.073$ ($n = 32$)

persons living in the Kristiansand area in southern Norway. The persons have no connection to the cobalt refining industry, and thus the results can be regarded as normal values for the population in the area.

In Table 4 we show the cobalt concentration in plasma from 10 persons, which in their work are exposed to water soluble $CoCl_2$.

The recovery of cobalt following digestion and extraction was tested by adding Co-60 to 10 plasma samples as well as to pure aqueous solutions. The average yield for the 10 plasma samples was better than 97% compared to the direct extraction of aqueous solutions. The results from the recovery test are shown in Table 5.

Table 4. Cobalt concentration in plasma (µg/l) from 10 cobalt refinery workers exposed to water soluble $CoCl_2$

A	14.5
B	13.5
C	10.2
D	4.3
E	1.5
F	2.3
G	6.5
H	24.7
I	13.7
J	5.7
Average	9.7 ± 7.1

Table 5. Recovery (%) of cobalt-60 after digestion and extraction

Sample 1	97.7
Sample 2	96.8
Sample 3	95.2
Sample 4	98.5
Sample 5	97.5
Mean ± SD 97.1 ± 1.24%, CV = 1.28%	

Discussion

Neutron activation, atomic absorption, arc spectroscopy and colorimetry have been used for the determination of cobalt in plasma from normal subjects.

The results reported, however, vary widely and values from 0.02 µg/l to 1,100 µg/l [4–24] have been reported.

From a medical point of view there is no reasonable explanation to this wide variation in results. Different exposure from air, food and drinking water will of course result in some variation from one geographical area to another. It appears to us, however, that the main reason for this discrepancies in results must be due to analytical difficulties. Contamination from the ambient air as well as from the reagents and glassware are also potential sources of error. Therefore, to obtain the lowest possible detection limit and to be able to analyze cobalt in plasma from normal subjects, it is important that contamination is reduced to a minimum and that reagent blanks are run through the whole procedure.

For this method we estimate 0.06 µg/l to be a realistic limit of detection. This figure can vary somewhat, dependent on the purity of the reagents and glassware used. From Fig. 1 it can be seen that the reagent blank is a substantial part of the absorption signal, and that the detection limit could be improved by further purification of the acids and reagents.

Barfoot et al. [5] used chloroform as organic phase. For two reasons we preferred to use MIBK instead of chloroform:

a) Our experience with chloroform is that it generates a background signal when atomized in a graphite furnace, and hence a background correction is needed. We observed no background signal when MIBK is used and thus the use of background corrector can be avoided.

b) The density of the MIBK phase is less than for the aqueous phase, and will locate on the top of the sample. This makes it possible to withdraw sample aliquots for atomic absorption analysis when a test tube is used without penetration or discarding the water phase.

Delves et al. [6] used APDC as chelating agent and found 100 % extraction. They extracted at pH 2.8 – 3.3. We find it more convenient with regard to pH-adjustment to extract from a basic solution. By using pH 9 we can also use the same extract for analysis of other heavy elements in whole blood and plasma (for instant Ni in plasma).

The results obtained by this method are in agreement with those obtained by the neutron activation method reported by Versieck et al. [4]. The results obtained by Barfoot et al. [5] differ from these results by one order of magnitude.

The method described will be used for control purposes and for biological monitoring of cobalt exposure. Earlier experience with nickel exposure has shown that such monitoring is an excellent way to reveal bad working conditions and working habits.

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