The MAK Collection for Occupational Health and Safety

Lead and its compounds – Determination of δ-aminolevulinic acid in urine by HPLC with fluorescence detection

Biomonitoring Method – Translation of the German version from 2018

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Keywords: lead; δ-aminolevulinic acid; ALA; biomonitoring; urine; HPLC-FLD

Citation Note: Göen T, Weißflog L, Blaszkewicz M, Baumhoer G, Schettgen T, Dewes P, Hartwig A, MAK Commission. Lead and its compounds – Determination of δ-aminolevulinic acid in urine by HPLC with fluorescence detection. Biomonitoring Method – Translation of the German version from 2018. MAK Collect Occup Health Saf [Original edition. Weinheim: Wiley-VCH; 2018 Oct;3(4):2170-2185]. Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2026. https://doi.org/10.34865/bi743992e2218_w

Republished (online): 06 Mar 2026

Originally published by Wiley-VCH Verlag GmbH & Co. KGaA; https://doi.org/10.1002/3527600418.bi743992e2218

Manuscript completed: 24 Nov 2016

Published (online): 19 Oct 2018

The commission established rules and measures to avoid conflicts of interest.

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Biomonitoring Methods

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DOI: 10.1002/3527600418.bi743992e2218

Abstract

The working group "Analyses in Biological Materials" of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area verified the presented biomonitoring method.

The method described hereinafter permits the determination of δ-aminolevulinic acid (ALA) in urine as a biological marker of effect to assess exposure to lead. An indicator of such exposure is the increased urinary excretion of ALA caused by lead-induced inhibition of the enzyme δ-aminolevulinic acid dehydratase. The determination of ALA in urine is based on a condensation reaction of ALA with formaldehyde and acetylacetone yielding a pyrrolizine derivate, which can be sensitively detected using fluorescence detection. 6-Amino-5-oxohexanoic acid is used as an internal standard. Calibration standards are prepared in pooled urine and processed in the same way as the samples to be analysed.

The method was extensively validated and the reliability data were confirmed by two independent laboratories, which have established and cross-checked the whole procedure.

Keywords

lead; δ-aminolevulinic acid; ALA; urine; biomonitoring; Analyses in Biological Materials; HPLC; fluorescence detection

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Lead and its compounds – Determination of δ-aminolevulinic acid in urine by HPLC with fluorescence detection

Matrix:	Urine
Hazardous substances:	Lead and its compounds
Analytical principle:	Liquid chromatography with fluorescence detection
Completed in:	November 2016

Overview of the parameters that can be determined with this method and the corresponding hazardous substances:

Hazardous substance	CAS	Parameter	CAS
Lead	7439-92-1	δ-Aminolevulinic acid (5-Amino-4-oxopentanoic acid)	106-60-5
Inorganic lead compounds, i.a.			
Lead(II) carbonate	598-63-0		
Lead(II) oxide	1317-36-8		
Lead(II) sulphate	7446-14-2		
Lead(II) sulphide	1314-87-0		

Summary

The method described hereinafter permits the determination of δ-aminolevulinic acid (ALA) in urine as a biological marker of effect to assess exposure to lead. This is due to an increased urinary excretion of ALA caused by lead-induced inhibition of the enzyme δ-aminolevulinic acid dehydratase. The determination of ALA in urine is based on a condensation reaction of ALA with formaldehyde and acetylacetone yielding a pyrrolizine derivate, which can be sensitively detected using fluorescence detection. 6-Amino-5-oxohexanoic acid is used as an internal standard. Calibration standards are prepared in pooled urine and processed in the same way as the samples to be analysed.

Reliability data of the method

δ-Aminolevulinic acid (ALA)

Within-day precision:	Standard deviation (rel.)	s_w = 5.95% or 3.40%
	Prognostic range	u = 14.1% or 8.03%
	at a spiked concentration of 0.25 or 0.95 mg ALA per litre urine and where n = 8 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 7.2% or 6.5%
	Prognostic range	u = 16.4% or 14.8%
	at a spiked concentration of 5.7 or 11.7 mg ALA per litre urine and where n = 10 determinations	
Accuracy:	Recovery rate (rel.)	r = 108 or 114%
	at a spiked concentration of 0.25 or 0.95 mg ALA per litre urine and where n = 10 determinations	
Detection limit:	10 µg ALA per litre urine	
Quantitation limit:	30 µg ALA per litre urine	

General information on δ-Aminolevulinic acid (ALA) as a biomarker of lead exposure

Lead and its inorganic compounds (except for lead arsenate and lead chromate) are classified by the Commission as Category 2 carcinogens as well as Category 3A germ cell mutagens. Hence, no MAK value can be derived for lead [DFG 2017]. For a summary of the toxicological properties of lead, please refer to the respective MAK Value Documentations of the Commission [Greim 2000, translated; Greim 2004; Greim 2007, translated].

δ-Aminolevulinic acid (ALA) is an endogenous substance, which is formed in the haematopoietic system. The enzyme δ-aminolevulinic acid dehydratase catalyses the formation of porphobilinogen, which is an intermediate in the biosynthesis of the haem pigment, from aminolevulinic acid. Internal lead exposure inhibits the enzyme δ-aminolevulinic acid dehydratase and thereby interferes with haem biosynthesis. Besides, this results in an accumulation of ALA in the organism and thus in an increased urinary excretion of ALA. The measured urinary ALA concentration is usually also an indicator of the extent of the exposure [Lehnert and Henschler 1989]. In contrast to standard exposure parameters, the determination of ALA in urine represents a biological effect parameter. In 2001, however, the BAT value for ALA in urine was withdrawn as the determination of ALA was no longer deemed recommendable for methodological and practical reasons (i.a. due to the long latency period, the unspecificity and the poor detection sensitivity of the former methods) [Lehnert and Greim 2001, translated]. The then valid BAT value was 15 mg ALA per litre urine, while a lower value of 6 mg ALA per litre urine was derived for women of child-bearing age [Lehnert and Henschler 1989]. Since then, a valid as-

sessment value for the urinary ALA level in Germany has not been available. In the case of lead exposure, it is thus generally recommended to determine blood lead concentrations, for which valid limit and reference values have been derived [Drexler and Hartwig 2014]. Nevertheless, the determination of the urinary ALA excretion enables the specific diagnosis of one of the most sensitive biological effects of lead exposure.

In other countries, such as Austria, it is still common practice to determine ALA in urine in the case of lead exposure and there are also assessment values for this parameter. It is hence necessary to develop and validate an efficient and robust analytical method for the determination of ALA in urine.

The conversion reaction of ALA applied in this method was first described by Okayama et al. [1988]. In detail, ALA reacts with two acetylacetone molecules and one formaldehyde molecule to form a pyrrolizine derivative (2,6-diacetyl-1,5-dimethyl-7-(2-carboxyethyl)-3H-pyrrolizine), which can be detected fluorimetrically. The underlying chemical reaction was described back in 1890 by the German chemist Arthur Hantzsch. Nevertheless, it was Kajiwara et al. [1993] who first elucidated the structure of the ALA derivative. Figure 1 shows the reaction of ALA to the pyrrolizine derivative.

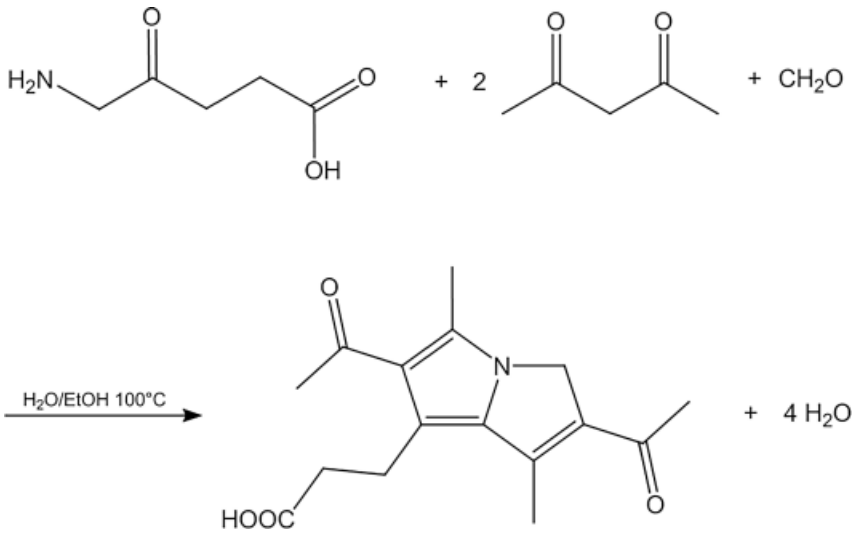

Figure 1 Conversion of ALA to a pyrrolizine derivative.

Contents

1 General principles

The method described hereinafter permits the determination of δ-aminolevulinic acid (ALA) in urine as a biological marker of effect to assess exposure to lead. This is due to an increased urinary excretion of ALA caused by lead-induced inhibition of the enzyme δ-aminolevulinic acid dehydratase. The determination of ALA in urine is based on a condensation reaction of ALA with formaldehyde and acetylacetone yielding a pyrrolizine derivate, which can be sensitively detected using fluorescence detection. 6-Amino-5-oxohexanoic acid is used as the internal standard. Calibration standards are prepared in pooled urine and processed in the same way as the samples to be analysed.

2 Equipment, chemicals and solutions

2.1 Equipment

- HPLC system comprising a pump, autosampler and fluorescence detector (e.g. Merck HITACHI)

- HPLC column: LiCrospher® PAH LiChroCART® 250-3, 250 x 3 mm, 5 µm (e.g. Merck)
- Vortex mixer (e.g. Scientific Industries)
- Water bath, thermostated (e.g. GFL)
- Ultrasonic bath
- Microlitre pipettes, variably adjustable from 10 to 100 µL as well as from 100 to 1000 µL (e.g. Eppendorf)
- Various pipettes (e.g. Eppendorf)
- Various volumetric flasks and beakers (e.g. VWR)
- 8 mL vials with screw caps and PTFE-coated inner septa (e.g. Brand)
- 1.8 mL vials with screw caps and PTFE-coated inner septa (e.g. VWR)

2.2 Chemicals

Unless otherwise specified, all chemicals must be at least p. a. grade.

- *δ*-Aminolevulinic acid, hydrochloride (e.g. Merck, No. 1.24802)
- 6-Amino-5-oxohexanoic acid, hydrochloride (e.g. MolPort, No. 021-780-066)
- Acetylacetone, for synthesis (e.g. Merck, No. 8.00023)
- Formaldehyde, 37% (e.g. Merck, No. 1.04003)
- Methanol (e.g. Carl Roth, No. 7342.1)
- Acetic acid, 100% (e.g. Merck, No. 1.00063)
- Ethanol, absolute, denatured with 1% methyl ethyl ketone (e.g. Merck, No. 1.00974)
- Deionised water

2.3 Solutions

Formaldehyde solution (10%)

After placing exactly 54 mL of deionised water into a 100 mL volumetric flask, 20 mL of the 37% formaldehyde solution are slowly added.

Acetylacetone solution (15%)

6 mL acetylacetone are mixed with 4 mL ethanol and 30 mL deionised water in a 50 mL volumetric flask. The solution is then placed into an ultrasonic bath for some minutes for complete dissolution of acetylacetone.

2.4 Internal Standard

Stock solution I (800 mg/L)

20 mg 6-amino-5-oxohexanoic acid (hydrochloride) are weighed exactly into a 20 mL volumetric flask and dissolved in deionised water. The flask is then filled to the mark with deionised water. Taking into account the molar ratio to hydrochloride, the concentration of the stock solution I of the internal standard is 800 mg/L.

Stock solution II (80 mg/L)

2 mL of the stock solution I of the internal standard are pipetted into a 20 mL volumetric flask, which is then filled to the mark with deionised water.

Working solution (5 mg/L)

0.5 mL of the stock solution II of the internal standard are pipetted into a 20 mL volumetric flask, which is then filled to the mark with deionised water.

The solutions are stored at –20°C and are stable under these conditions for at least 1 year without significant losses.

2.5 Calibration standards

Stock solution (1000 mg/L)

12.8 mg δ-aminolevulinic acid (hydrochloride) are weighed exactly into a 10 mL volumetric flask and dissolved in deionised water. The flask is then filled to the mark with deionised water. Taking the molar ratio to hydrochloride into account, the concentration of the stock solution is 1000 mg/L.

Working solution (100 mg/L)

1 mL of the stock solution is pipetted into a 10 mL volumetric flask. The flask is then filled to the mark with pooled urine.

The solutions are stored at –20°C and are stable under these conditions for at least 6 months without significant losses.

Calibration standards

The calibration standard solutions are prepared in pooled urine of individuals occupationally non-exposed to lead. To prepare the pooled urine, spot urine samples from the individuals are collected in a suitable container, mixed thoroughly and stored at –20°C.

Calibration standards in a concentration range from 0.05 to 10 mg/L ALA are prepared by diluting the working solution of δ-aminolevulinic acid with pooled urine according to the pipetting scheme given in Table 1. The pooled urine used is also included as a blank value.

3 Specimen collection and sample preparation

The urine samples are collected in sealable plastic containers and stored at –20°C. In this way, the urine samples are stable for at least 6 months.

Prior to analysis, the samples are thawed at room temperature and mixed thoroughly. 25 µL of the sample are pipetted into a brown 8 mL screw cap vial and 25 µL of the working solution of the internal standard, 1.75 mL of the 15% acetylacetone solution as well as 225 µL of the 10% formaldehyde solution are added. Subsequently, the vial is sealed and the solution is mixed thoroughly on a Vortex

mixer for about 30 s. For derivatisation, the sample is then incubated at 100°C for 60 min using a water bath. To stop derivatisation, the sample is cooled in a light-protected box filled with ice water for about 30 min.

After cooling, 1 mL of each sample solution is transferred to brown 1.8 mL screw cap vials and analysed using HPLC with fluorescence detection. At each stage it has to be ensured that the samples are preferably stored in the dark.

Table 1 Pipetting scheme for the preparation of calibration standards to determine ALA in urine.

Calibration standard	Volume of the spiking solution	Final volume of the calibration standard	Concentration ALA
	[µL]	[mL]	[mg/L]
0	0	10	0
1	5	10	0.05
2	10	10	0.10
3	25	10	0.25
4	50	10	0.50
5	100	10	1.0
6	200	10	2.0
7	500	10	5.0
8	1000	10	10.0

4 Operational parameters

Analysis was performed using a liquid chromatography system (HPLC) coupled with an autosampler, a fluorescence detector and a data processing system.

4.1 Liquid chromatography

Column:	Column packing:	LiCrospher® PAH LiChroCART®250-3
	Inner diameter:	3.0 mm
	Particle size:	5 µm
	Length:	250 mm
Separation principle:	Reversed Phase	
Mobile phase:	Methanol/water/glacial acetic acid (50/50/1, V/V/V)	
Flow rate:	0.4 mL/min	
Injection volume:	10 µL	
Eluent program:	isocratic	

4.2 Fluorescence detection

Excitation:	363 nm
Detection:	473 nm

All other parameters have to be optimised in accordance with the manufacturer's specifications.

5 Analytical determination

For analytical determination of the urine samples prepared as described in Section 3, 10 µL of each derivatised sample solution are injected into the HPLC system. Identification of the analyte is based on the retention time (t_R (ALA) = 6.7 min; t_R (internal standard) = 8.3 min). A quality control sample and a reagent blank value consisting of deionised water are included in each analytical run. The peak areas of the analyte and of the internal standard are recorded.

The retention times given are intended to be a rough guide only. Users of the method must ensure proper separation performance of the analytical column used influencing the resulting retention behaviour of the analytes. Figure 2 (in the Appendix) shows as an example a chromatogram of a standard solution in pooled urine.

6 Calibration

The calibration standards prepared in pooled urine (see Section 2.5) are processed in the same way as the urine samples according to Section 3 and analysed as stipulated in Sections 4 and 5. The calibration graph is obtained by plotting the quotient of the peak area of the analyte and of the internal standard against the concentration of the respective calibration standard. The linearity of the calibration graph was tested and confirmed in a concentration range between the detection limit and an ALA concentration of 10 mg/L. Figure 3 (in the Appendix) shows an example of a calibration graph for ALA prepared in pooled urine.

7 Calculation of the analytical results

The analyte concentration in a sample is determined by dividing the peak area of the analyte by the peak area of the internal standard. The quotient thus obtained is entered in the calibration curve of Section 6. The intercept of the calibration graph reflects the physiological ALA background level in the pooled urine used for calibration and is not taken into account. Any reagent blank values which may be present are accounted for by subtraction. The analyte levels are obtained in mg/L.

8 Standardisation and quality control

Quality control of the analytical results is carried out as stipulated in the guidelines of the Bundesärztekammer (German Medical Association) and in a general chapter of the MAK Collection for Occupational Health and Safety Part IV: Biomonitoring Methods [Bader et al. 2010, translated; Bundesärztekammer 2014]. To check precision, a quality control sample with a known and constant ALA concentration is analysed within each analytical run. As material for quality control is not commercially available, it must be prepared in the laboratory.

To this end, pooled urine is spiked with a defined amount of ALA, aliquoted and stored at −20°C. The nominal value and the tolerance ranges of the quality control material are determined in a pre-analytical period (one analysis of the control material on each of 20 different days) [Bader et al. 2010, translated].

9 Evaluation of the method

The reliability of the method was proved by comprehensive validation and by implementation and validation of the procedure in two independent laboratories.

9.1 Precision

To determine within-day precision, pooled urine samples from occupationally non-exposed individuals were spiked with an ALA standard solution and then prepared, processed and analysed. The spiked ALA concentrations were 0.25 mg/L, 0.95 mg/L, 5.0 mg/L as well as 10 mg/L. The within-day precision data obtained by 8-fold determination of these urine samples are given in Table 2.

To determine day-to-day precision, the G-EQUAS control materials RV 53A and RV 53B were prepared, processed and analysed on eight different days. The obtained day-to-day precision data are presented in Table 3.

9.2 Accuracy

Recovery experiments were performed to determine the accuracy of the method. To this end, pooled urine from occupationally non-exposed individuals was spiked with ALA at concentrations of 0.25 mg/L and 0.95 mg/L, divided into eight aliquots and analysed. Taking into account the urinary background level (0.33 mg/L), the relative recovery rates given in Table 4 were obtained. Besides, the G-EQUAS (German External Quality Assessment Scheme) control materials RV 53A and RV 53B were prepared, processed and analysed ten times in a row. The established values were used to calculate the mean relative recovery.

Moreover, this method was repeatedly tested in external quality assessment schemes (G-EQUAS) for the determination of ALA in urine run by the German Society of Occupational and Environmental Medicine (Deutsche Gesellschaft für Arbeitsmedizin und Umweltmedizin; DGAUM). The results thus obtained by this method are summarised in Table 5.

Table 2 Within-day precision for the determination of ALA in urine (n = 8).

Material	Spiked concentration	Determined value	Standard deviation (rel.)	Prognostic range
	[mg/L]	[mg/L]	[%]	[%]
Spiked urine	0	0.33	–	–
	0.25	0.62	5.95	14.1
	0.95	1.45	3.40	8.0
	5.0	5.56	4.93	11.7
	10	10.4	6.24	14.8

Table 3 Day-to-day precision for the determination of ALA in urine (n = 10).

Material	Reference value	Determined value	Standard deviation (rel.)	Prognostic range
	[mg/L]	[mg/L]	[%]	[%]
RV 53A	5.7	5.18	7.23	16.4
RV 53B	11.7	11.4	6.54	14.8

9.3 Limits of detection and limits of quantitation

The detection limit was estimated on the basis of the 3-fold signal-to-noise ratio. The quantitation limit was determined analogously (9-fold signal-to-noise ratio).

Under the given conditions of sample preparation and analytical determination, the detection limit and quantitation limit for the determination of ALA in urine were 10 µg/L and 30 µg/L, respectively.

9.4 Sources of error

Studies have described that ALA is only stable in acidic aqueous solutions, while it is quickly degraded at physiological pH values [Elfsson et al. 1999; Gadmar et al. 2002]. This raises the question of the stability of the biomarker in urine and the possible necessity of urine acidification to stabilise ALA as described in a previous method developed by the working group [Angerer and Schaller 1978]. Elfsson et al. [1999] investigated the degradation of ALA in aqueous media at different temperatures and pH-values and found the degradation reaction to be dependent both on temperature and pH-value. However, the ALA degradation is a second-order reaction so that the half-life depends on the initial concentration. For their studies, Elfsson et al. [1999] used very high ALA concentrations of up to 10 g/L, which are irrelevant for human biomonitoring. At a concentration of 10 g ALA/L the half-life of ALA at pH 7.4 and 37°C is 3 h. However, at a concentration of 10 mg ALA/L, which may be expected in biomonitoring, the half-life is >120 d.

Table 4 Mean relative recovery rates for the determination of ALA in urine (n = 10).

Material	Spiked concentration	Determined value	Mean rel. recovery	Range
	[mg/L]	[mg/L]	[%]	[%]
Spiked urine	0.25	0.62	108	98.7–118
	0.95	1.45	114	108–121
RV 53A	5.7	5.2	93.2	79.6–105
RV 53B	11.7	11.4	99.2	88.4–105

Table 5 Results of the external quality assessment schemes for the determination of ALA in urine.

Material	Determined value	Nominal value (tolerance range)	Accuracy
	[mg/L]	[mg/L]	[%]
RV 53A	5.3	5.7 (4.5–6.9)	92.9
RV 53B	11.6	11.7 (10.2–13.2)	99.1
RV 54A	2.8	2.8 (2.2–3.4)	100
RV 54B	15.5	14.2 (12.1–16.3)	109
RV 55A	4.1	4.3 (3.4–5.2)	95.3
RV 55B	2.3	2.5 (1.9–3.1)	92.0

Tests on analyte stability performed by the developers of this method confirm this calculation. For stability testing, urine samples were spiked with different ALA concentrations and the pH-value was adjusted to pH 7 and pH 2, respectively. The samples were processed directly or after storage under specific conditions (+4°C and –20°C; for 5 days and 28 days). In the case of 28-day storage, no dependence of the ALA stability on the pH-value or storage temperature could be identified. However, the dependence of analyte degradation on the initial concentration described by the second-order degradation kinetics is quite apparent. Due to uncertainties caused by the single implementation of the storage tests, it is, nevertheless, recommended to acidify the urine samples to pH 2 and to store the urine samples and standard solutions to be analysed at –20°C.

The spiking solution of the internal standard should also be stored at –20°C as it shows certain degradation after some weeks when stored in the refrigerator (+4°C). Basically, even when stored at –20°C, the internal standard starts to degrade. In the case of repeated freeze-thaw cycles over a period of two years, about 30–40% of the internal standard was degraded.

A prolonged storage of processed urine samples at room temperature and under the influence of light has a detrimental effect on the analytical results. For this reason, the processed samples should always be stored in the dark and analysed as soon as possible.

During sample work-up, one of the external verifiers of the method encountered losses in some samples due to the high derivatisation temperature (100°C) combined with the volatility of acetylacetone, which led to unreproducible results. In this case, derivatisation in headspace vials with gas-tight crimp caps helped considerably to improve precision and accuracy of the method. The developers of this method also investigated derivatisation in headspace vials with gas-tight crimp caps. Therefore, standard solutions of ALA prepared in urine with a concentration of 10 mg/L were processed and analysed. However, at the laboratory of the developers of the method, no differences regarding recovery were found. Nevertheless, in case of poor method precision, users of this method should consider using crimp cap vials for derivatisation.

During the external method verification, it was observed that the method gave false high results in the case of one individual urine sample with an elevated creatinine level. The chromatogram of this urine sample, however, showed no visible interfering peaks in the range of the analyte and the internal standard. A closer examination revealed that not the high creatinine level but the use of an aged derivatisation reagent (4–5 weeks old) seems to be accountable for the observed excessive recovery rates. It is therefore advisable to use a derivatisation reagent that has possibly been freshly prepared on the very same day.

10 Discussion of the method

The method presented herein is based on the work of Okayama [1988] and permits the precise and sensitive determination of δ-aminolevulinic acid (ALA) in urine as a biological marker of effect to assess exposure to lead. The introduction of an internal standard has considerably improved the analytical validation data regarding linearity, recovery, precision and sensitivity compared to the methods previously published by the Commission.

Instruments used

Analysis was performed using a liquid chromatography system (HPLC) coupled with an autosampler, a fluorescence detector and a data processing system.

11 References

Angerer J, Schaller KH, Arbeitsgruppe Analysen in biologischem Material (1978) δ-Aminolävulinsäure. In: Pilz W, Angerer J, Schaller KH, Henschler D (Eds) Analytische Methoden zur Prüfung gesundheitsschädlicher Arbeitsstoffe, Band 2: Analysen in biologischem Material, 2. Lieferung, VCH, Weinheim
https://doi.org/10.1002/3527600418.bihememetabd0002a

Bader M, Barr D, Göen Th, Schaller KH, Scherer G, Angerer J, Arbeitsgruppe Analysen in biologischem Material (2010) Zuverlässigkeitskriterien einer analytischen Methode. In: Angerer J und Hartwig A (Eds): Analytische Methoden zur Prüfung gesundheitsschädlicher Arbeitsstoffe, Band 2: Analysen in biologischem Material, 19. Lieferung, Wiley-VCH, Weinheim
https://doi.org/10.1002/3527600418.bireliabd0019, translated
https://doi.org/10.1002/3527600418.bireliabe0012

Bundesärztekammer (2014) Richtlinien der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen. Dt Ärztebl 111: A1583–1618

DFG (Deutsche Forschungsgemeinschaft) (2017) List of MAK and BAT Values 2017, Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Report No. 53, Wiley-VCH, Weinheim
https://doi.org/10.1002/9783527812127

Drexler H, Hartwig A (Eds) (2014) Addendum zu Blei und seine Verbindungen (außer Bleiarsenat, Bleichromat und Alkylbleiverbindungen). Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte), Expositionsäquivalente für krebserzeugende Arbeitsstoffe (EKA), Biologische Leitwerte (BLW) und Biologische Arbeitsstoff-Referenzwerte (BAR), 21. Lieferung, Wiley-VCH, Weinheim
https://doi.org/10.1002/3527600418.bb743992d0021

Elfsson B, Wallin I, Eksborg S, Rudaeus K, Ros AM und Ehrsson H (1999) Stability of 5-aminolevulinic acid in aqueous solution. Eur J Pharm Sci 7(2): 87–91

Gadmar OB, Moan J, Scheie E, Ma LW und Peng Q (2002) Stability of 5-aminolevulinic acid in solution. J Photochem Photobiol B, 67(3): 187–193

Greim H (Ed.) (2000) Blei und seine anorganischen Verbindungen, außer Bleiarsenat und Bleichromat. Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 31. Lieferung, Wiley-VCH, Weinheim
https://doi.org/10.1002/3527600418.mb743992d0031, translated
https://doi.org/10.1002/3527600418.mb743992e0017

Greim H (Ed.) (2004) Blei und seine anorganischen Verbindungen (einatembare Fraktion). Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 38. Lieferung, Wiley-VCH, Weinheim
https://doi.org/10.1002/3527600418.mb743992d0038

Greim H (Ed.) (2007) Blei und seine anorganischen Verbindungen (einatembare Fraktion). Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 43. Lieferung, Wiley-VCH, Weinheim
https://doi.org/10.1002/3527600418.mb743992d0043, translated
https://doi.org/10.1002/3527600418.mb743992e0025

Kajiwara M, Hara K, Takatori K, Matsumoto K (1993) Revised structure of a delta-aminolevulinic acid derivative. Clin Chem 39(9): 1867–1871

Lehnert G, Henschler D (Eds) (1989) Blei und seine Verbindungen (außer Bleiarsenat, Bleichromat und Alkylbleiverbindungen). Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte) und Expositionsäquivalente für krebserzeugende Arbeitsstoffe (EKA), 4. Lieferung, Wiley-VCH, Weinheim
https://doi.org/10.1002/3527600418.bb743992d0004

Lehnert G, Greim H (Eds) (2001) Blei und seine Verbindungen (außer Bleiarsenat, Bleichromat und Alkylbleiverbindungen). Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte) und Expositionsäquivalente für krebserzeugende Arbeitsstoffe (EKA), 10. Lieferung, Wiley-VCH, Weinheim
https://doi.org/10.1002/3527600418.bb743992d0010, translated
https://doi.org/10.1002/3527600418.bb743992e0004a

Okayama A (1988) Fluorimetric determination of urinary delta-aminolevulinic acid by high-performance liquid chromatography and post-column derivatization. J Chromatogr 426(2): 365–369

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12 Appendix

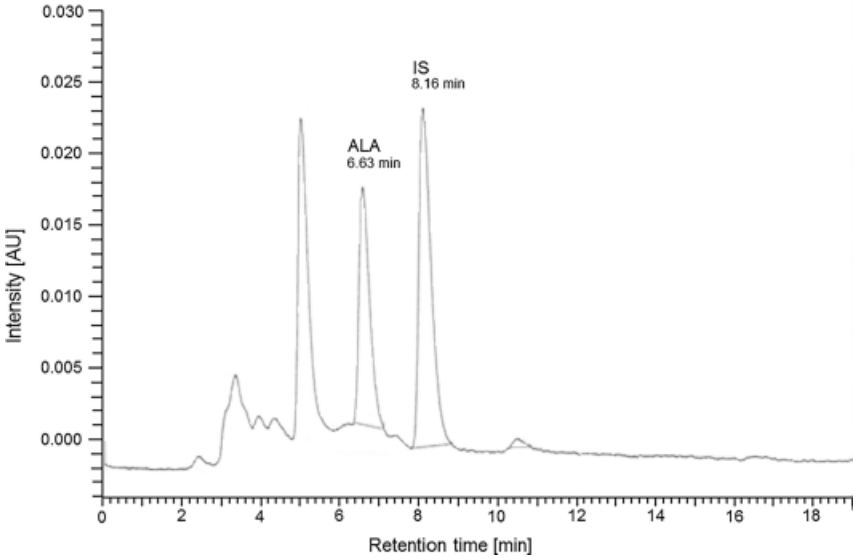

Figure 2 Chromatogram of a standard solution with an ALA concentration of 0.1 mg/L in pooled urine.

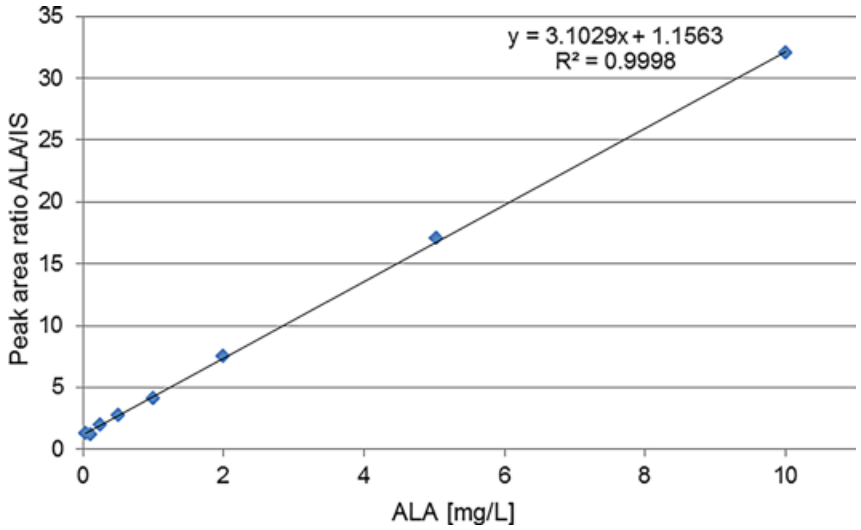

Figure 3 Calibration graph for ALA in the concentration range from 0.5 to 10 mg/L.