Method for the determination of aromatic amines

German Social Accident Insurance

Expert Committee Raw Materials and Chemical Industry
Subcommittee Hazardous Substances
Analytical Subcommittee of the Chemistry Board of Experts[*]

Recognized analysis procedures for carcinogenic mutagenic or substances toxic to reproduction

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This method has been tested and recommended for the determination of aromatic amines in the air at workplaces by the German Social Accident Insurance.

Both personal and stationary sampling can be performed for the assessment of workplaces.

Sampling is carried out with a pump and adsorption on a filter impregnated with acid. Analysis is performed by gas chromatography after desorption.

Name	CAS No.	Molar mass
Aniline; aminobenzene	62-53-3	93.13
2,4-Dimethylaniline; 2,4-xylidine	95-68-1	121.18
2,5-Dimethylaniline; 2,5-xylidine	95-78-3	121.18
2,6-Dimethylaniline; 2,6-xylidine	87-62-7	121.18
4-Ethoxyaniline; p-phenetidine	156-43-4	137.18
2-Methoxyaniline; o-anisidine	90-04-0	123.15
3-Methoxyaniline; m-anisidine	536-90-3	123.15
4-Methoxyaniline; p-anisidine	104-94-9	123.15
2-Methylaniline; o-toluidine	95-53-4	107.16
3-Methylaniline; m-toluidine	108-44-1	107.16
4-Methylaniline; p-toluidine	106-49-0	107.16

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Summary

The method permits the determination of aniline, 4-ethoxyaniline, methoxyanilines, methylanilines and dimethylanilines concentrations – abbreviated to amines in this method – in working areas averaged over the sampling time after personal or stationary sampling.

Principle:	A defined volume of air is drawn through a filter holder equipped with a filter impregnated with acid using a pump. The adsorbed amines are then desorbed from the filter with a mixture of acetonitrile/ammonia solution and determined by a mass selective detector (MSD) after separation by means of gas chromatography.
Limit of quantification:	Absolute: 0.2 to 1.6 ng per amine Relative: 0.003 to 0.026 mg/m^3 of an amine for an air sample volume of 240 L, 4 mL of desorption solution and an injection volume of 1 µL (see also Section 5.2).
Measurement range:	0.02 to 0.6 mg/m^3 for an air sample volume of 240 mL (1 mL/min flow rate; sampling duration 4 hours).
Selectivity:	A high degree of selectivity is achieved due to the gas chromatographic separation and the use of a mass selective detector (MSD).
Advantages:	Personal and selective measurements are possible.
Disadvantages:	No indication of peak concentrations.
Apparatus:	Pump, flow meter, filter holder with acid-impregnated filter, gas chromatograph with mass selective detector (MSD).

Detailed description of the method

Contents

1
Equipment, chemicals and solutions

1.1
Equipment

For sampling:

- Sampling pump, suitable for a flow rate of 1 mL/min, e.g. PP5, from Gilian, supplied by Haan & Wittner, 71288 Friolzheim, Germany
- Flow meter, e.g. Gilibrator, from Gilian
- GSP sampling system with a suction cone for a volumetric flow of 1 L/min, e.g. from GSM, 41469 Neuss, Germany
- Glass fibre filter, diameter 37 mm (e.g. MN 85/90 BF type, from Macherey and Nagel, 52355 Düren, Germany)

For sample preparation and analytical determination:

- Flatbed shaker
- Analytical balance
- Adjustable piston pipette, e.g. Multipette pro, from Eppendorf, 22339 Hamburg, Germany
- Microlitre syringes with volumes from 10 to 250 µL
- Volumetric flasks, 5 mL, 10 mL, 250 mL, 1000 mL
- Glass vials, 5 mL
- Gas chromatograph with mass selective detector (MSD)
- Filter holder with spacer rings (for validation), e.g. Aerosol Analysis Monitors, Order No. M000037A0, from Millipore, 65824 Schwalbach, Germany

1.2
Chemicals and solutions

- Aniline, purity 99.5%, e.g. from Sigma-Aldrich, 82024 Taufkirchen, Germany
- 2,4-Dimethylaniline, purity 98%, e.g. from Sigma-Aldrich
- 2,5-Dimethylaniline, purity 99%, e.g. from Sigma-Aldrich
- 2,6-Dimethylaniline, purity 99%, e.g. from Sigma-Aldrich
- 4-Ethoxyaniline, purity 98%, e.g. from Sigma-Aldrich
- 2-Methylaniline, purity 99%, e.g. from Sigma-Aldrich
- 3-Methylaniline, purity 99%, e.g. from Sigma-Aldrich
- 4-Methylaniline, purity 99.7%, e.g. from Sigma-Aldrich
- 2-Methoxyaniline, purity 99%, e.g. from Sigma-Aldrich
- 3-Methoxyaniline, purity 97%, e.g. from Sigma-Aldrich
- 4-Methoxyaniline, purity 99%, e.g. from Sigma-Aldrich
- 4-Propylaniline, purity 98% (internal standard (ISTD)), e.g. from Sigma-Aldrich
- Ammonia solution, 25%, e.g. from Merck, 64293 Darmstadt, Germany
- Acetonitrile, Chromasolv, purity 99.9%, e.g. from Sigma-Aldrich
- Methanol, p.a., e.g. from Merck
- Sulphuric acid 0.5 mol/L, e.g. from Merck
- Helium 5.0, purity 99.999% (carrier gas) for gas chromatograph

Solvent mixture:	Mixture of acetonitrile/ammonia solution (95:5 v/v) 50 mL of ammonia solution are placed into a 1000 mL volumetric flask, and the flask is filled to the mark with acetonitrile and shaken. The solvent mixture is stable for at least six months.
Desorption solution:	Solution of approx. 36 mg 4-propylaniline/L solvent mixture Several millilitres of solvent mixture are placed into a 250 mL volumetric flask. Then 10 µL of propylaniline (density 0.919 g/mL) are added using a pipette, the flask is filled to the mark with solvent mixture and shaken.
Stock solutions:	Solutions of approx. 3 mg of each amine per mL of the desorption solution (see Table 1). The amines listed in Table 1 are weighed exactly to the nearest 0.1 mg into a 10 mL volumetric flask. The volumetric flask is then filled to the mark with desorption solution and shaken.

Table 1 Masses and concentrations of the amines in the stock solution

Substance	**Mass [mg]**	**Concentration [mg/mL]**
Aniline	29.94	3.0
2,4-Dimethylaniline	32.64	3.2
2,5-Dimethylaniline	32.22	3.2
2,6-Dimethylaniline	37.82	3.7
4-Ethoxyaniline	27.98	2.7
2-Methoxyaniline	29.95	3.0
3-Methoxyaniline	32.58	3.2
4-Methoxyaniline	31.60	3.1
2-Methylaniline	26.93	2.7
3-Methylaniline	27.24	2.7
4-Methylaniline	30.10	3.0

Calibration solutions: Solutions of approx. 1.4 to 37 mg of amine per L of the desorption solution (see Table 2).

The six volumes of the stock solution shown in Table 2 are each pipetted into a separate 10 mL volumetric flask, the flask is filled to the mark with the desorption solution and shaken.

Table 2 Concentrations of the amines in the calibration solutions in mg/L

Calibration solution	**1**	**2**	**3**	**4**	**5**	**6**
Stock solution added [µL]	**5**	**20**	**40**	**60**	**80**	**100**
Aniline	1.49	5.96	11.9	17.9	23.8	29.8
2,4-Dimethylaniline	1.60	6.40	12.8	19.2	25.6	32.0
2,5-Dimethylaniline	1.59	6.38	12.8	19.1	25.5	31.9
2,6-Dimethylaniline	1.87	7.49	15.0	22.5	30.0	37.4
4-Ethoxyaniline	1.37	5.48	11.0	16.5	21.9	27.4
2-Methoxyaniline	1.48	5.93	11.9	17.8	23.7	29.7
3-Methoxyaniline	1.58	6.32	12.6	19.0	25.3	31.6
4-Methoxyaniline	1.56	6.26	12.5	18.8	25.0	31.3
2-Methylaniline	1.33	5.33	10.7	16.0	21.3	26.7
3-Methylaniline	1.35	5.39	10.8	16.2	21.6	27.0
4-Methylaniline	1.50	6.00	12.0	18.0	24.0	30.0

A concentration range from approx. 0.02 to 0.6 mg/m^3 of each amine (see Table 3) is covered by these solutions based on an air sample volume of 240 L.

Table 3 Converted concentrations of the amines in the calibration solutions in mg/m^3 (based on an air sample volume of 240 L)

Calibration solution	1	2	3	4	5	6
Aniline	0.025	0.099	0.20	0.30	0.40	0.50
2,4-Dimethylaniline	0.027	0.11	0.21	0.32	0.43	0.53
2,5-Dimethylaniline	0.027	0.11	0.21	0.32	0.43	0.53
2,6-Dimethylaniline	0.031	0.12	0.25	0.37	0.50	0.62
4-Ethoxyaniline	0.023	0.091	0.18	0.27	0.37	0.46
2-Methoxyaniline	0.025	0.099	0.20	0.30	0.40	0.49
3-Methoxyaniline	0.026	0.105	0.21	0.32	0.42	0.53
4-Methoxyaniline	0.026	0.104	0.21	0.31	0.42	0.52
2-Methylaniline	0.022	0.089	0.18	0.27	0.36	0.44
3-Methylaniline	0.022	0.090	0.18	0.27	0.36	0.45
4-Methylaniline	0.025	0.10	0.20	0.30	0.40	0.50

1.3

Preparation of the impregnated filter

The glass fibre filters are immersed in the 0.5 molar sulphuric acid, placed on a watch glass and air-dried overnight. The impregnated filters are stored in sealed glass dishes at room temperature and are stable under these conditions for at least six months.

2

Sampling

An acid-impregnated glass fibre filter is inserted into the GSP sampling system and connected to the pump. A flow rate of 1 L/min is set. The definition of the inhalable dust is fulfilled by this volumetric flow according to DIN EN 481 [1]. The pump and the sampling system are either worn by a person during sampling or they are used for stationary sampling. The method was tested up to a sample volume of 240 L (corresponding to a sampling time of 4 hours). After sampling, the flow rate must be checked for constancy. If the deviation from the adjusted flow rate is greater than ± 5%, it is advisable to reject the measurements (for details see BGI/GUV-I 505-0 [2] “General Part”, Section 3). The filter holder is subsequently removed from the sampling system and sealed tightly.

3
Analytical determination

3.1
Sample preparation and analysis

The glass fibre filter is transferred into a 5 mL glass vial for preparation. After addition of 4 mL of desorption solution, the vial is immediately sealed and shaken for 30 minutes on a flatbed shaker. Then an aliquot of the supernatant solution (sample solution) is transferred into an autosampler vial.

In order to ensure that the desorption solution and the filter used do not contain any interfering impurities, an acid-impregnated filter per sample series is extracted – as described above – with 4 mL of desorption solution (blank solution).

1 µL each of the sample solution and the blank solution are injected into the gas chromatograph and a chromatogram is recorded as described below (Section 3.2). The quantitative evaluation is performed according to the internal standard method using the peak areas of the selected amine and that of 4-propylaniline as the internal standard.

3.2
Operating conditions for gas chromatography

The characteristics of the method stated in Section 5 were obtained under the following operating conditions:

Apparatus:	HP 6890A gas chromatograph with mass selective detector (MSD 5973 Network) and split/splitless injector, from Agilent
Separation column:	Quartz capillary, stationary phase VF-WAX-MS cross-linked, internal diameter 0.25 mm, length 30 m, film thickness 0.5 µm, from Varian
Temperatures:	Injector: 250 °C Oven with temperature program: Initial temperature: 100 °C Heating rate I: 25 °C/min until 170 °C Heating rate II: 3 °C/min until 190 °C Heating rate III: 25 °C/min to 240 °C, 5 min isothermal
Injection:	Split ratio 1:10
Injection volume:	1 µL
Carrier gas:	Helium 5.0
MSD conditions:	Ionisation type: Electron impact (EI) Ionisation energy: 70 eV Measurement mode: SIM/scan (mass range: 29 – 250 amu)

Table 4 SIM evaluation masses (m/z)

Substance	Target	Qualifier 1	Qualifier 2
Aniline	93	66	65
2,4-Dimethylaniline	121	120	106
2,5-Dimethylaniline	121	120	106
2,6-Dimethylaniline	121	120	106
4-Ethoxyaniline	108	109	137
2-Methoxyaniline	108	123	80
3-Methoxyaniline	123	94	93
4-Methoxyaniline	108	123	80
2-Methylaniline	106	107	77
3-Methylaniline	106	107	77
4-Methylaniline	106	107	77
4-Propylaniline (ISTD)	106	135	–

4
Evaluation

4.1
Calibration

In each case 1 µL of the six calibration solutions of the amines described in Section 1.2 are injected into the gas chromatograph.

The ratios of the peak areas of the amines with respect to the peak areas of 4-propylaniline (ISTD) are plotted against the corresponding mass ratios obtained for the calibration solutions. The calibration function is obtained by means of regression analysis.

4.2
Calculation of the analytical result

The peak areas of the amines and of 4-propylaniline are determined from the chromatograms recorded, the quotient is formed and the corresponding value for the mass in the sample solution in µg is calculated from the calibration function. The concentration by weight of each amine in the air sample in mg/m^3 is calculated according to Equation (1):

$$c = \frac{m}{V \cdot \eta} \qquad (1)$$

where:

c is the mass concentration of the relevant amine in the air sample in mg/m^3

m is the mass of the amine in the sample solution in µg obtained from the corresponding calibration function

V is the air sample volume in L

η is the recovery (see Section 5.1)

If the measured concentrations of an individual amine are outside the calibration range, then a suitable dilution with desorption solution must be prepared and the analysis must be carried out again.

5
Reliability of the method

5.1
Precision and recovery

The precision in the minimum measurement range according to DIN EN 482 [3] and the recovery were determined for three different concentrations. The validation solution was prepared as shown in Table 5:

Validation solution:	Solution of approx. 2 to 37 mg amine per mL of methanol The masses of the amines for each solution stated in Table 5 were weighed to exactly the nearest 0.1 mg into a 5 mL volumetric flask. Then the flask is filled to the mark with methanol and shaken.

Millipore filter holders with spacer rings holding one non-impregnated filter and two acid-impregnated filters were used to perform the validation. A microlitre syringe was used to spike the non-impregnated glass fibre filter with 5, 50 and 100 µL of the validation solution. The filters impregnated with sulphuric acid (separated by a spacer ring) are connected downstream. Laboratory air was drawn through this combination at a flow rate of 1 L/min for four hours at an ambient temperature of 23 °C and a relative humidity of approx. 55%. The preparation and analytical determination were carried out as described in Section 3. Based on an air sample volume of 240 L, the spiked masses corresponded with the validation concentrations are given in Table 5.

Table 5 Concentrations of the amines in the validation solution and during validation

Substance	Mass [mg]	Concentration [mg/mL]	Validation concentration		
			c_1	c_2	c_3
			[mg/m^3]	[mg/m^3]	[mg/m^3]
Aniline	186.2	37.05	0.77	7.72	15.4
2,4-Dimethylaniline	24.1	4.77	0.10	0.994	1.99
2,5-Dimethylaniline	30.2	5.98	0.13	2.25	2.49
2,6-Dimethylaniline	25.8	5.10	0.11	1.06	2.12
4-Ethoxyaniline	26.9	5.27	0.11	1.01	2.20
2-Methoxyaniline	28.6	5.66	0.12	1.18	2.36
3-Methoxyaniline	14.8	2.86	0.06	0.597	1.19
4-Methoxyaniline	11.9	2.36	0.05	0.493	0.99
2-Methylaniline	15.4	3.05	0.06	0.636	1.27
3-Methylaniline	26.0	5.15	0.11	1.07	2.15
4-Methylaniline	25.2	5.04	0.11	1.05	2.10

The recoveries and relative standard deviations shown in Table 6 were obtained for the individual concentrations when the procedure described above was carried out six times in each case. In this case no breakthrough to the second acid-impregnated filter could be detected.

Table 6 Precision and recovery for the validation of the amines

Substance	Recovery				Relative standard deviation [%]			
	c_1	c_2	c_3	Mean	c_1	c_2	c_3	Mean
Aniline	0.97	0.98	0.97	**0.97**	1.3	0.5	1.7	**1.2**
2,4-Dimethylaniline	0.96	0.99	0.98	**0.98**	1.9	0.6	2.3	**1.6**
2,5-Dimethylaniline	0.95	0.98	0.98	**0.97**	1.8	0.6	1.9	**1.4**
2,6-Dimethylaniline	0.94	0.97	0.96	**0.96**	1.4	0.2	2.2	**1.3**
4-Ethoxyaniline	1.04	0.99	1.04	**1.02**	1.0	0.9	2.3	**1.4**
2-Methoxyaniline	0.95	0.98	0.98	**0.97**	1.0	0.4	2.1	**1.2**
3-Methoxyaniline	0.96	0.98	0.99	**0.98**	2.5	0.7	2.1	**1.7**
4-Methoxyaniline	1.04	1.01	1.03	**1.03**	4.5	0.9	2.0	**2.5**
2-Methylaniline	0.96	0.98	0.97	**0.97**	3.5	0.5	1.9	**1.9**
3-Methylaniline	0.99	0.99	1.00	**0.99**	1.9	0.7	1.8	**1.5**
4-Methylaniline	0.99	0.99	0.99	**0.99**	1.3	0.6	1.6	**1.2**

5.2
Limit of quantification

The limit of quantification was determined according to DIN 32465 [4] by means of a 10-point calibration in the lower concentration range of approx. 5 to 50 µg/m^3 (based on an air sample volume of 240 L) for a statistical certainty of 99% and a relative uncertainty of 33.3 % (k = 3).

The absolute limits of quantification for the individual amines are between 0.2 and 1.6 ng. At an air sample volume of 240 L, a desorption volume of 4 mL and an injection volume of 1 µL, this is equivalent to relative limits of quantification of 0.003 to 0.026 mg/m^3 (see Table 7).

Table 7 Limits of quantification of the amines

Substance	**Limits of quantification**	
	absolute [ng]	**relative [µg/m^3]**
Aniline	0.4	6
2,4-Dimethylaniline	0.2	3
2,5-Dimethylaniline	0.3	5
2,6-Dimethylaniline	0.2	4
4-Ethoxyaniline	1.6	26
2-Methoxyaniline	0.4	7
3-Methoxyaniline	1.0	17
4-Methoxyaniline	1.1	18
2-Methylaniline	0.6	10
3-Methylaniline	0.5	8
4-Methylaniline	0.3	5

5.3
Selectivity

A high degree of selectivity is achieved due to the gas chromatographic separation and the use of a mass selective detector (MSD).

5.4
Expanded uncertainty

The expanded uncertainty was estimated taking all relevant influencing factors into consideration as stipulated in DIN EN 482 [3]. The uncertainty of the entire method and thus also of the analytical result consists principally of the uncertainty contributions of the air sampling (e.g. air sample volume, deviation from the sampling convention) and the analytical preparation (extraction agent volume, scatter of the calibration function, fluctuations in the recovery and the reproducibility).The expanded uncertainty is between 21% and 23%, depending on the amine and concentration.

5.5
Storage stability

The loaded sample filter with the adsorbed substances can be stored at room temperature without losses for at least 14 days.

References

1. DIN EN 481 (1993) Workplace atmospheres – Size fraction definitions for measurement of airborne particles. Beuth Verlag, Berlin, Germany
2. BGI/GUV-I 505-0 (2003) A. General Part, Carl Heymanns Verlag, Cologne, Germany, http://publikationen.dguv.de/dguv/pdf/10002/bgi505-0.pdf
3. DIN EN 482 (2012) Workplace atmospheres – General requirements for the performance of procedures for the measurement of chemical agents. Beuth Verlag, Berlin, Germany
4. DIN 32645 (2008) Chemical analysis – Decision limit, detection limit and determination limit under repeatability conditions – Terms, methods, evaluation. Beuth Verlag, Berlin

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Appendix

Chromatogram of a calibration mixture

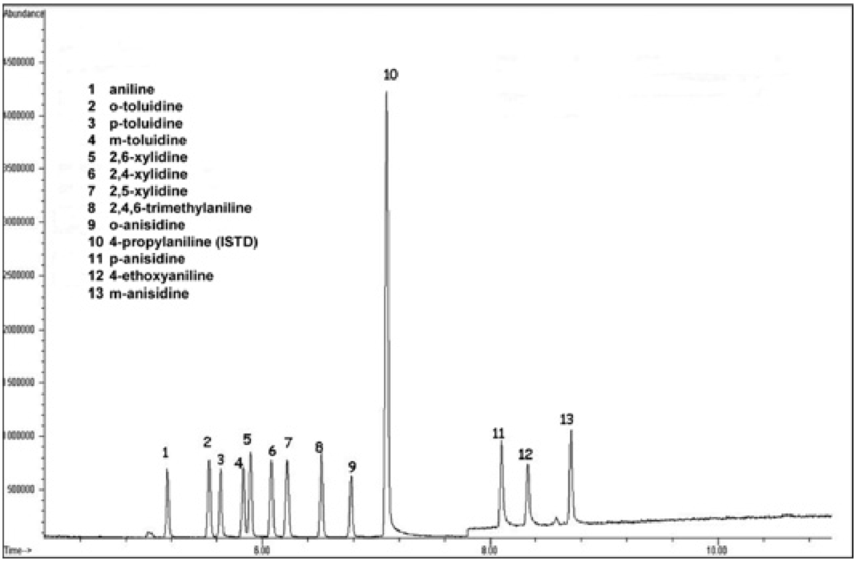

Fig. 1 Gas chromatogram of selected amines at concentrations of approx. 80 to 100 µg/m^3.