

HPLC method for the determination of aromatic amines released from water-colours under physiological conditions

Autor(en): **Bürgi, Christoph / Bollhalder, Rita / Otz, Theresa**

Objektyp: **Article**

Zeitschrift: **Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene = Travaux de chimie alimentaire et d'hygiène**

Band (Jahr): **88 (1997)**

Heft 3

PDF erstellt am: **10.08.2024**

Persistenter Link: <https://doi.org/10.5169/seals-982328>

Nutzungsbedingungen

Die ETH-Bibliothek ist Anbieterin der digitalisierten Zeitschriften. Sie besitzt keine Urheberrechte an den Inhalten der Zeitschriften. Die Rechte liegen in der Regel bei den Herausgebern.

Die auf der Plattform e-periodica veröffentlichten Dokumente stehen für nicht-kommerzielle Zwecke in Lehre und Forschung sowie für die private Nutzung frei zur Verfügung. Einzelne Dateien oder Ausdrucke aus diesem Angebot können zusammen mit diesen Nutzungsbedingungen und den korrekten Herkunftsbezeichnungen weitergegeben werden.

Das Veröffentlichen von Bildern in Print- und Online-Publikationen ist nur mit vorheriger Genehmigung der Rechteinhaber erlaubt. Die systematische Speicherung von Teilen des elektronischen Angebots auf anderen Servern bedarf ebenfalls des schriftlichen Einverständnisses der Rechteinhaber.

Haftungsausschluss

Alle Angaben erfolgen ohne Gewähr für Vollständigkeit oder Richtigkeit. Es wird keine Haftung übernommen für Schäden durch die Verwendung von Informationen aus diesem Online-Angebot oder durch das Fehlen von Informationen. Dies gilt auch für Inhalte Dritter, die über dieses Angebot zugänglich sind.

HPLC Method for the Determination of Aromatic Amines Released from Water-Colours under Physiological Conditions

Key words: Water-colours, Aromatic amines, HPLC, Collaborative trial, Toys

Christoph Bürgi, Rita Bollhalder and Theresa Otz
Kantonales Laboratorium Basel-Stadt, Basel

Participants of the collaborative trial:
Ernst Rutschmann, Kantonales Laboratorium Zürich, Zürich;
Jürg Noser, Kantonales Laboratorium Basel-Landschaft, Füllinsdorf;
Alain Etournaud, Laboratoire cantonal vaudois, Epalinges

Introduction

Depending on the purity of raw materials and completeness of manufacturing processes, azo dyes may be contaminated by free aromatic amines. Water-colours made with azo dyes may therefore contain traces of primary aromatic amines. Some of these compounds are harmful or even carcinogenic. When using water-colours, children are likely to swallow them, mostly by sucking the brush. In Switzerland legal restrictions have been stipulated for the maximum release of aromatic amines from water-colours for children (1). Extraction under physiological conditions (50-fold excess of hydrochloric acid 0.07 mol/l as gastric juice simulant, 2 h at 37° C) is prescribed.

Legal restrictions distinguish between two kinds of aromatic amines: a) «the sum of aromatic amines, quantified as aniline» (maximum value: 50 mg/kg), b) «the sum of benzidine, 2-aminonaphthaline, 4-aminobiphenyl and other amines with the same physiological behaviour» (maximum value: 5 mg/kg). Simple determination of the sum of all aromatic amines, for example by diazotation followed by colorimetry, is therefore not sufficient to control the maximum values. Hence a provisional HPLC-method is given in the Swiss Food Manual, beside a provisional colorimetric sum-method (2). It describes the determination of the four aromatic amines aniline, benzidine, 2-naphthylamine and 4-aminobiphenyl. The aim of this work

was to refine and validate the method for selective identification and quantitation of as many other aromatic amines as possible. The choice of the additional compounds corresponds to indications of the WHO (3, 4) and the list of MAK-values (5). The toxicological data of the selected compounds are listed in table 1. All of these primary aromatic amines are potential structural elements of azo-dyes.

The refined method was developed starting from the mentioned provisional HPLC-method and a publication on the determination of impurities in food dyes (6). It allows the simultaneous determination of all of the 11 amines which are mentioned in table 1. After separation with reversed-phase HPLC, detection is carried out with a UV-detector, confirmation with a DAD (Diode Array Detector).

The main difference between the presented method and a recent German publication on textile testing (7) lies in sample preparation (acid extraction versus reductive cleavage of azo dyes) which influences clean up procedures and detection limits.

The presented method was tested by 4 laboratories. The results of the collaborative trial are reported in this paper.

Table 1. Toxicological data

Compound	according to (4)	according to (5)
2,4-diaminoanisole	cancerogenous (animal)	group IIIA2
2,4-diaminotoluene	cancerogenous (animal)	group IIIA2
aniline	possible cancerogenous (animal)	group IIIB
benzidine	cancerogenous (human)	group IIIA1
p-toluidine	not mentioned	group IIIB
o-dianisidine	cancerogenous (animal)	group IIIA2
o-tolidine	cancerogenous (animal)	group IIIA2
1-naphthylamine	possible cancerogenous (animal)	not mentioned
2-naphthylamine	cancerogenous (human)	group IIIA1
4-aminobiphenyl	cancerogenous (human)	group IIIA1
3,3'-dichlorbenzidine	cancerogenous (animal)	group IIIA2

Method

Materials and instruments

Coffee- or laboratory-mill, water bath with magnetic stirrer and immersion thermostat (e.g. IKA-RTM 5 «basic» complete for 5 stirring points), Extrelut® columns 20 ml refillable Merck-Art. 11737, filter 0.45 µm (e.g. Nylon Acrodisc 13, SKAN AG, 4009 Basel, prod.-nr. 4426), polyethylene syringes 1 ml.

HPLC-system (e.g.: Waters 600 MS pump, Waters 484 MS detector, Waters Model Code 600 column oven, ERMA Tokyo ERC 3811 degasser, Maxima chromatography software, Waters 990 photodiode array detector).

HPLC-column: LiChrosorb RP-18, 250×4 mm, 5 µm, precolumn 11×4 mm, e.g. Knauer GmbH, Berlin.

Reagents

Extrelut® 20 refill Merck 11738, pH-test strips e.g. Merck 9531. All other chemicals and solvents were of analytical or chromatography grade.

Reference materials

2,4-diaminoanisole (4-methoxy-1,3-phenyldiamine-sulfate-hydrate) Aldrich 18,332-6 (CAS-Nr. 615-05-4), 2,4-diaminotoluene (2,4-toluyldiamine) pract. >98% Fluka 33360 (CAS-Nr. 95-80-7), aniline >99,5% Merck 1261 (CAS-Nr. 62-53-3), benzidine purum p.a. >98% Fluka 12115 (CAS-Nr. 92-87-5), p-toluidine puriss p. a. >99% Fluka 89630 (CAS-Nr. 106-49-0), o-dianisidine (3,3'-dimethoxybenzidine) purum p.a. >99% Fluka 33430 (CAS-Nr. 119-90-4), o-tolidine (3,3'-dimethylbenzidine) puriss p.a. >98% Fluka 89580 (CAS-Nr. 119-93-7), 1-naphthylamine purum >98% Fluka 70731 (CAS-Nr. 134-32-7), 2-naphthylamine 95% Aldrich A6, 640-5 (CAS-Nr. 91-59-8), 4-aminobiphenyl purum >98% Fluka 07130 (CAS-Nr. 92-67-1), 3,3'-dichlorbenzidine-dihydrochloride (86-92% dihydrochloride) Pfaltz&Bauer D13700 612-3-9 or SIGMA D-9886 (>99% dihydrochloride) (CAS-Nr. 91-94-1).

Procedure

Eluant

Mix 662 g phosphate buffer pH 6 (dissolve 20.7 g sodium dihydrogenphosphate-monohydrate in about 800 ml of water, add 5 ml sodium hydroxide solution 5 mol/l and fill to 1 l with water) and 273 g acetonitrile.

Note: Weigh the parts of buffer and acetonitrile for best reproducibility.

HPLC-parameters

Temperature: 40 °C, sensitivity: 0.020 aufs, flow rate: 1.00 ml/min, detection wavelength: 17 min at 240 nm and 23 min at 278 nm, measuring time: 40 min, injection volume: 20 µl test solution. DAD: Wavelength range: 220–300 nm, wavelength of chromatogram: 240 nm, measuring time: 0–40 min, interval: 2.69 sec, y-scale: -0.005–0.04 AU, resolution: 2 nm.

Calibration solutions

Stock solutions: Prepare 50 ml solutions of 100 mg of each reference compound (dichlorobenzidine-dihydrochloride and 4-aminobiphenyl: 200 mg each) in methanol. Stability: Several months stored at 4°C in the dark, except 2,4-diaminoanisole which decomposes after one day.

Calibration solutions: Pipette 1 ml of each stock solution in the same flask and dilute to 20 ml with methanol (dilution 1). Pipette 1 ml of dilution 1 in a flask and dilute to 100 ml with eluant (dilution 2). Prepare dilution 1 and 2 daily and store them in the dark.

Note: No difference in calibration could be observed by preparing dilution 2 with a mixture of eluant and hydrochloric acid 0.07 mol/l instead of only eluant.

Calibration

Inject 2 µl (standard 1), 5 µl (standard 2), 10 µl (standard 3), 20 µl (standard 4) and 40 µl (standard 5) of dilution 2. The concentrations of the amines in dilution 2 are 1 to 2 ng/µl depending on weight-in quantity.

Note: Calibration can also be done by injecting equal volumes of different dilutions.

Sample preparation

Crush solid water-colour samples in a mortar if necessary. Grind at least 20 g of (crushed) water-colour to powder with a mill and mix it well. Weigh exactly approximately 0.5 g of this powder. Weigh tube colours directly. Stir in a 50 ml Erlenmeyer flask with 20 ml hydrochloric acid 0.07 mol/l for 1 minute. Control pH with a test strip. If the value is higher than 1.5, correct to 1.5 with hydrochloric acid 6 mol/l. Add hydrochloric acid 0.07 mol/l to a final volume of 25 ml. Stir the suspension for 1 hour at 37 °C in the dark. Keep it one more hour under the same conditions without stirring. Centrifuge the suspension, take exactly 20 ml of the supernatant and pour it to 1 ml of sodium hydroxide solution 5 mol/l in a porcelain evaporating dish. Stir and control pH with a test strip. If the value is lower than 10, correct to 10 with sodium hydroxide solution. Add one portion of Extrelut® 20 refill, homogenise well and wait for 20 minutes. Fill the column tightly with the whole mixture by knocking. Elute successively with 100 ml of ether and 50 ml of dichloromethane. Collect the elute in a 250 ml round bottomed flask that contains 4 ml of hydrochloric acid 0.07 mol/l. Evaporate at 40 °C to 3–4 ml. Blow out the residual solvent with nitrogen for 30 seconds. Transfer the aqueous residue quantitatively in a flask and dilute with eluant to 10 ml. Filter with a 1 ml polyethylene syringe through a 0.45 µm-filter before injection.

Analysis of chromatograms

Determine the peak areas at 240 or 278 nm as described under «HPLC-parameters» (an example of a chromatogram is given in figure 1a). The peak allocation results from retention times and UV-spectra. A DAD-chromatogram with UV-spectra is given in figure 1b.

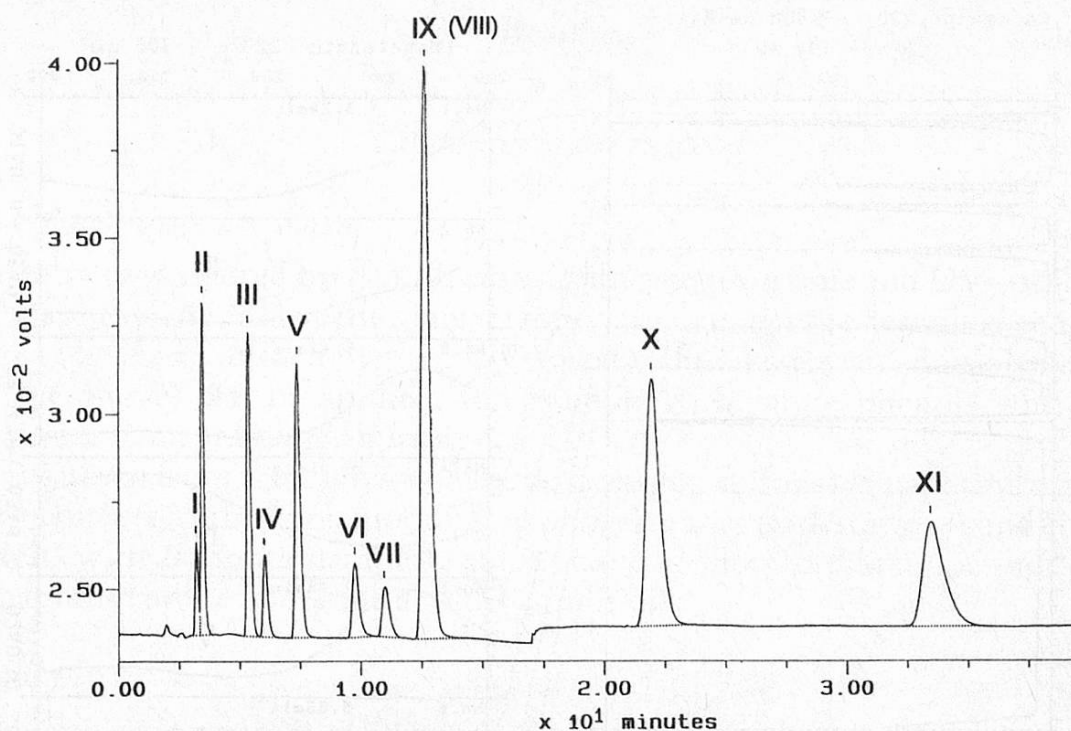


Fig. 1a. HPLC of standard 4 (2,4-diaminoanisole (I), 2,4-diaminotoluene (II), aniline (III), benzidine (IV), p-toluidine (V), o-dianisidine (VI), o-tolidine (VII), 1-naphthylamine (VIII, not injected, same retention time as IX), 2-naphthylamine (IX), 4-aminobiphenyl (X), 3,3'-dichlorbenzidine (XI), conditions see text)

Additional cleaning step (2)

Add 15 ml of hydrochloric acid 1 mol/l to the aqueous residue. Wash twice with 5 ml dichloromethane. Basify the aqueous phase to pH 12 with approximately 5 ml of sodium hydroxide solution 5 mol/l. Reextract 3 times with 5 ml ether. Combine the etherous extracts, add 2 ml hydrochloric acid 0.1 mol/l and evaporate at 40 °C to 1–2 ml. Blow out the residual solvent with nitrogen for 30 seconds. Transfer the aqueous residue quantitatively in a flask and dilute with eluant to 10 ml. Filter with a 1 ml polyethylene syringe through a 0.45 µm-filter before injection.

Calculation

Recalculate the content of the original sample with the following formula:

$$\frac{x \cdot 10 \cdot 25}{20 \cdot E} = \text{mg/kg}$$

x = concentration of amine in the injected solution determined from the calibration curve in µg/ml, E = weighed portion of sample in g, 10 = dissolution to 10 ml before injection, 25 = total amount of hydrochloric acid for extraction in ml, 20 = amount of extract used for analysis in ml.

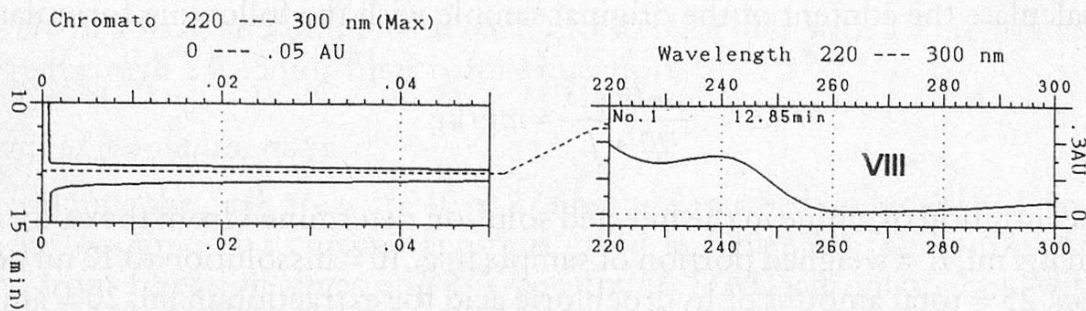
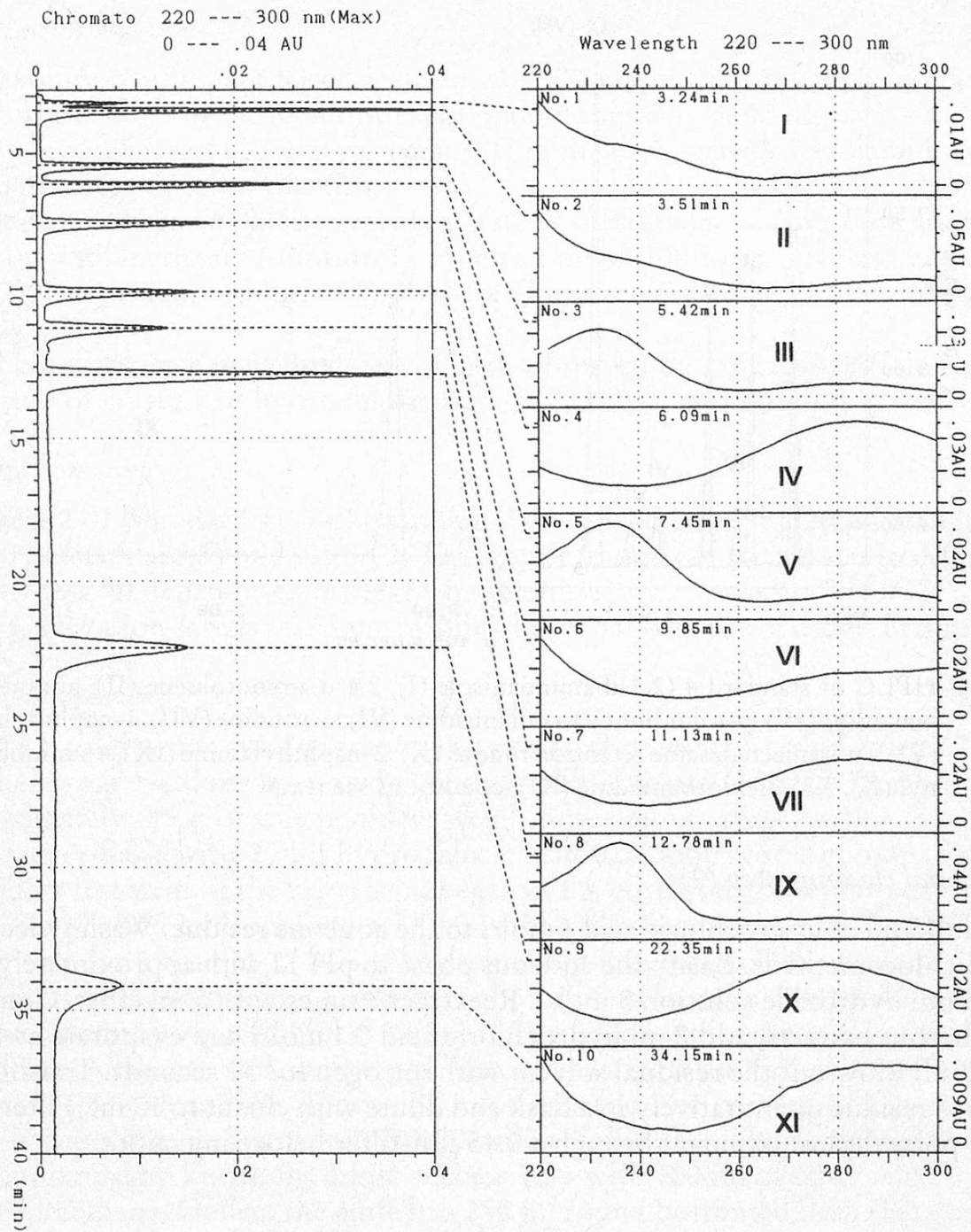


Fig. 1b. Figure 1a with diode array detection (DAD conditions: see text)

Results

Validation of the method

Selectivity

Selectivity is assured by identification with retention time and UV-spectrum. 1- and 2-naphthylamine have the same retention times under the described conditions. They can be distinguished by their UV-spectra. If necessary an additional cleaning step (see above) can be applied, for example to separate phenols with similar UV-spectra from aromatic amines.

It is known from the analysis of textile dyes that sulfonated naphthylamines are able to convert to the corresponding naphthylamines under acidic conditions (8). Our tests with 2-naphthylamine-1-sulphonic acid showed that it does not convert to 2-naphthylamine under the applied conditions.

Linearity

The calibration curves of all mentioned aromatic amines are linear in the range described under «Calibration».

Detection limit

The detection limits are listed in table 2. They have been determined by comparison of the threefold height of the noise with the height of the lowest standard peaks by linear extrapolation.

Table 2. Detection limits (explanations: see text)

Compound	detection limit	detection limit per kg of water-colour
2,4-diaminoanisole	0.12 ng	0.15 mg
2,4-diaminotoluene	0.09 ng	0.11 mg
aniline	0.10 ng	0.13 mg
benzidine	0.53 ng	0.66 mg
p-toluidine	0.14 ng	0.18 mg
o-dianisidine	0.44 ng	0.55 mg
o-tolidine	0.59 ng	0.74 mg
1-naphthylamine	not determined	
2-naphthylamine	0.05 ng	0.06 mg
4-aminobiphenyl	0.23 ng	0.29 mg
3,3'-dichlorbenzidine	0.50 ng	0.63 mg

Note: The detection wavelengths described under «HPLC-parameters» are preset by technical reasons. For individual compounds the detection limits can be enhanced by detection at their maximum absorption wavelength. A possibility of enhancing the detection limit by a factor of two is to carry out two extractions and to combine the two resulting «Extrelut[®]-extracts» before evaporation.

Quantitation limit

Normally we defined the quantitation limit as the concentration of standard 1. For data on repeatability of standard 1: see «Precision».

Recovery

See «Collaborative trial»

Precision

Repeatabilities of peak areas are listed in tables 3a and 3b. Their relative standard deviations range from 2 to 19% for standard 1 and 0.1 and 1.2% for standard 5.

Collaborative trial

The robustness of this method was tested in a collaborative trial with 4 laboratories. In the Swiss Food Manual a collaborative trial with 4 participants is defined as a «preliminary test for the method (Methodenvorprüfung)» (9). Analytical methods which passed this test, are published in the Swiss Food Manual as «recom-

Table 3a. Repeatability of the area values of standard 1

Compound	\bar{x} area ($n = 3$)	s
2,4-diaminoanisole	2748	519 (18.9%)
2,4-diaminotoluene	7597	155 (2.0%)
aniline	9175	73 (0.8%)
benzidine	2731	43 (1.6%)
p-toluidine	10812	245 (2.3%)
o-dianisidine	3637	239 (6.6%)
o-tolidine	2609	313 (12.0%)
1-naphthylamine	not determined	
2-naphthylamine	37071	1319 (3.6%)
4-aminobiphenyl	27678	771 (2.8%)
3,3'-dichlorbenzidine	18080	1715 (9.5%)

Table 3b. Repeatability of the area values of standard 5

Compound	\bar{x} area (n = 3)	s
2,4-diaminoanisoole	53938	424 (0.8%)
2,4-diaminotoluene	145338	607 (0.4%)
aniline	173983	843 (0.5%)
benzidine	56613	295 (0.5%)
p-toluidine	210559	366 (0.2%)
o-dianisidine	74360	703 (1.0%)
o-tolidine	55664	645 (1.2%)
1-naphthylamine	not determined	
2-naphthylamine	719326	888 (0.1%)
4-aminobiphenyl	548360	2774 (0.5%)
3,3'-dichlorbenzidine	351602	4103 (1.2%)

mended methods». A spiked and an unspiked sample of ground solid water colours were prepared for this purpose.

Preparation of samples

Approximately 200 g solid water-colours of different tones were ground as described above (sample 1). In preliminary tests no aromatic amines could be determined (detection limits: see table 2). An aliquot of sample 1 was then spiked with all of the aromatic amines mentioned in table 1, except 1-naphthylamine (sample 2). The amines were added as a methanol diluted stock solution (dilution factor: 2) in a mortar under an argon atmosphere. Sample 2 was thoroughly mixed under these conditions. The added amounts are given under «Recovery». Both samples were stored in the dark under argon at -18°C . Every participant got an aliquot of sample 1 and 2 to test. Typical chromatograms of sample 1 and 2 are shown in figures 2a and 2b.

To control the quality (recovery) of sample preparation and analysis, every participant had to prepare a special amine standard solution and to treat 1 ml of it like a sample. Preparation: Pipette 1 ml of dilution 1 (see «Calibration solutions») in a flask and dilute to 10 ml with hydrochloric acid 0.07 mol/l.

Results of sample 1 (unspiked sample)

Each laboratory analyzed the sample three times. Not one of the mentioned amines were determined (detection limits: see table 2). Only laboratory 3 supposed 1.9 mg/kg ($s = 1.8$ mg/kg) of 2,4-diaminoanisoole and reported 0.6 mg/kg ($s = 0.09$ mg/kg) of p-toluidine.

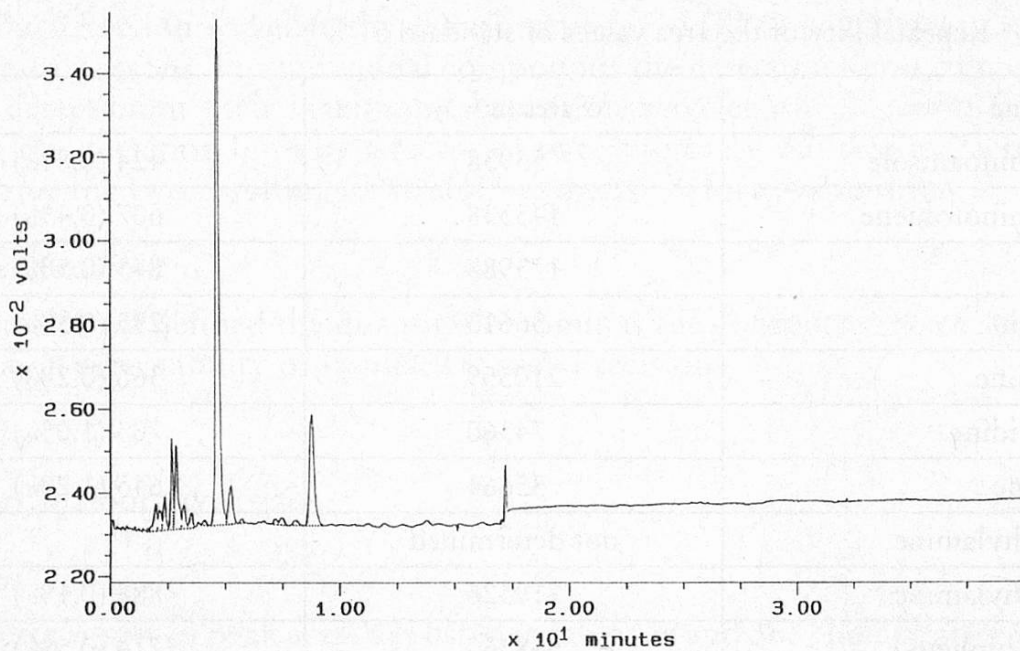


Fig. 2a. HPLC of collaborative trial sample 1 (unspiked; conditions: see text)

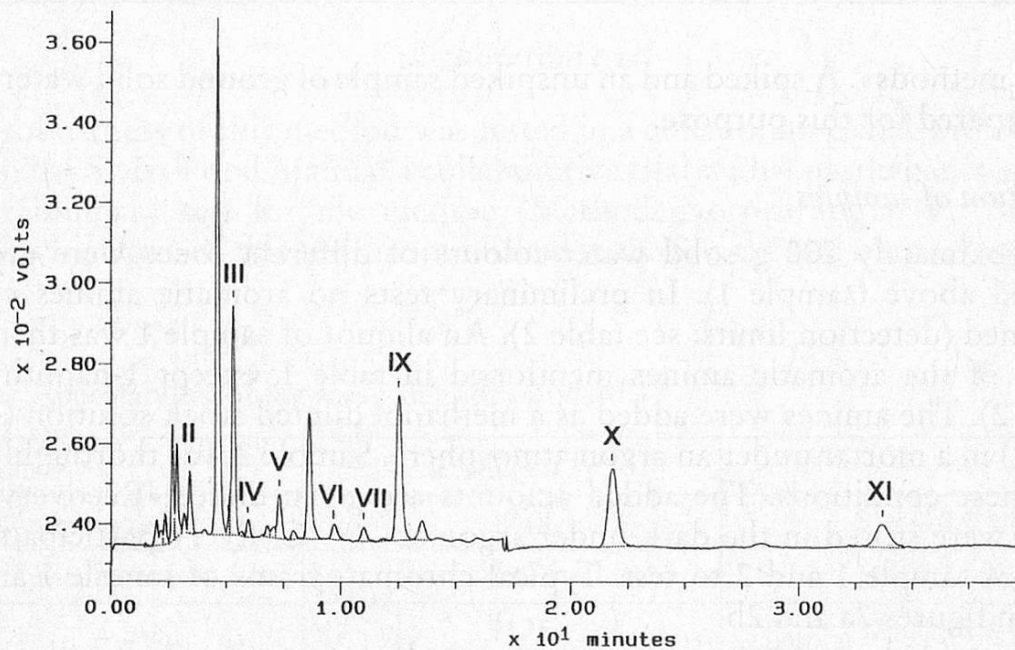


Fig. 2b. HPLC of collaborative trial sample 2 (2,4-diaminotoluene (II), aniline (III), benzidine (IV), p-toluidine (V), o-dianisidine (VI), o-tolidine (VII), 2-naphthylamine (IX), 4-aminobiphenyl (X), 3,3'-dichlorbenzidine (XI), conditions see text)

Results of sample 2 (spiked sample)

Each laboratory analyzed the sample three times. Data are displayed in table 4 and in figure 3. Recovery rates (see below) have not been taken into account for the calculation.

Nine of the ten added amines were detected and quantitated by all laboratories. Three laboratories supposed 2,4-diaminoanisoole, but it could only be confirmed by

Table 4. Collaborative trial, results of sample 2 in mg/kg (added amounts: see table 5)

	2,4-diaminoaniso- sole	2,4-diaminoto- luene	aniline	benzi- dine	p-tolui- dine	o-dianisi- dine	o-toli- dine	1-naph- thyl- amine	2-naph- thyl- amine	4-amino- biphenyl	3,3'-di- chlor- benzidine	Σ (without 2,4- diaminoaniso- sole and 3,3'-dich- lorbenzidine)
lab. 1	<i>n.d.</i>	3.84	14.33	2.97	3.51	4.18	3.96	<i>n.d.</i>	3.61	8.94	4.90	45.34
lab. 1	<i>n.d.</i>	3.94	14.04	3.13	3.57	4.52	4.06	<i>n.d.</i>	3.67	9.33	3.55	46.26
lab. 1	<i>n.d.</i>	4.84	14.81	3.22	3.69	5.57	4.59	<i>n.d.</i>	3.84	9.36	5.03	49.92
lab. 2	2.3?	2.7	13.8	3.4	3.5	4.8	4.5	<i>n.d.</i>	3.1	10.0	11.6*	45.8
lab. 2	2.3?	2.7	13.0	3.1	3.3	4.4	4.3	<i>n.d.</i>	3.0	8.8	10.9*	42.6
lab. 2	2.3?	2.4	12.9	3.2	3.0	4.8	4.2	<i>n.d.</i>	3.0	8.1	11.1*	41.6
lab. 3	1.80?	3.04	13.33	3.29	3.36	6.07	4.69	<i>n.d.</i>	3.60	10.68	3.25	48.06
lab. 3	1.41?	4.68	11.78	3.57	3.65	7.65	5.23	<i>n.d.</i>	4.16	11.92	3.48	52.64
lab. 3	3.31?	5.34	13.54	3.89	3.46	6.86	5.49	<i>n.d.</i>	3.93	11.25	6.03	53.76
lab. 4	1.90	4.12	12.59	3.29	3.43	4.40	4.84	<i>n.d.</i>	3.29	9.68	2.13	45.64
lab. 4	1.80	3.92	12.25	3.33	3.71	4.70	3.68	<i>n.d.</i>	3.33	9.07	2.07	43.99
lab. 4	2.02	5.08	12.84	25.80	3.91	5.79	8.22	<i>n.d.</i>	3.75	11.13	4.94	76.52**
\bar{x}		3.77	13.31	3.31	3.47	5.27	4.50		3.52	9.74	3.81	46.87
<i>s</i>		0.97	0.91	0.25	0.20	1.14	0.54		0.39	1.14	1.41	3.89
<i>s</i> (%)		25.6%	6.9%	7.6%	5.9%	21.6%	12.1%		11.1%	11.7%	37.1%	8.3%
<i>m</i>		3.92	13.33	3.29	3.50	4.80	4.50		3.60	9.36	3.52	45.80
\bar{x} (robust)		3.98	13.24	3.29	3.51	4.63	4.31		3.48	9.34	3.49	46.29
<i>r</i>		0.86	1.27	0.49	0.55	1.34	1.36		0.28	1.74	0.75	7.02

	2,4-diaminoanisole	2,4-diaminoluene	aniline	benzidine	p-toluidine	o-dianisidine	o-toluidine	1-naphthylamine	2-naphthylamine	4-amino-biphenyl	3,3'-dichlorbenzidine	Σ (without 2,4-diaminoanisole and 3,3'-dichlorbenzidine)
<i>r</i> (%)		21.5%	9.6%	14.9%	15.6%	28.8%	31.4%		7.9%	18.6%	21.5%	15.2%
<i>R</i>		2.53	2.76	0.73	0.56	1.18	1.16		1.28	2.39	4.44	13.51
<i>R</i> (%)		63.4%	20.9%	22.3%	15.8%	25.4%	26.9%		36.9%	25.5%	127.5%	29.2%

*Outlier (90–95%) of the 4 laboratory mean values according to Grubbs (10)

**Outlier (>99%) of the 12 Σ according to Grubbs (10)

n.d. = not detectable, lab. = laboratory

r = repeatability, *R* = reproducibility according to (9)

italics: not used for statistics

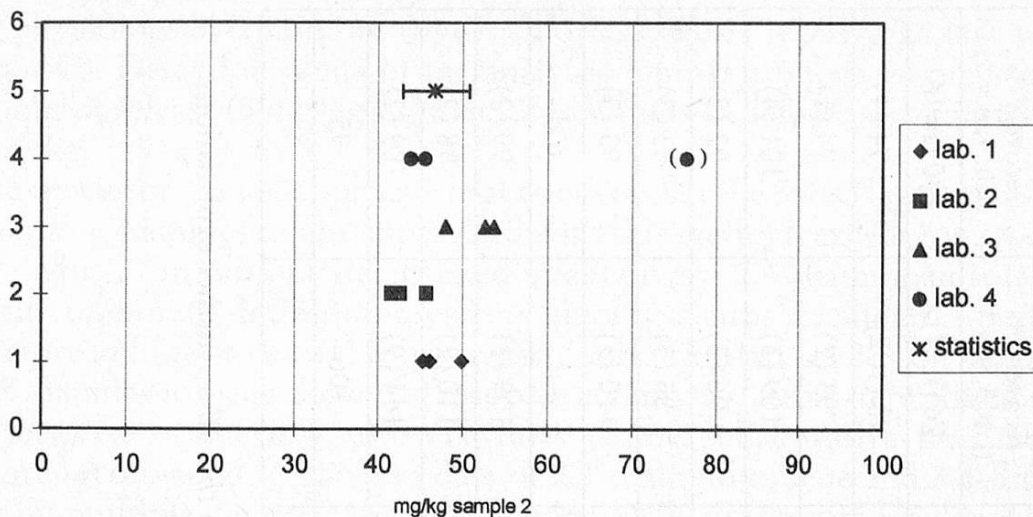


Fig. 3. Graphical display of the results of the collaborative trial. Sum values of all amines (except 2,4-diaminoanisole and 3,3'-dichlorbenzidine, see text) found in sample 2 (spiked). In brackets: outlier

laboratory 4. The content of 3,3-dichlorbenzidine found by laboratory 2 is very high and can be treated as an outlier (90–95% according to *Grubbs* (10)). Even without the results of laboratory 2, the results for 3,3-dichlorbenzidine indicate a bad reproducibility. The values for 2,4-diaminoanisole and 3,3-dichlorbenzidine have therefore not been used for the analysis of the sum of all aromatic amines.

Recovery

The recovery of aromatic amines depends strongly on the composition of the water colours and should theoretically be determined for each sample separately. Yet this is not necessary for the present analytical question, because Swiss legal restrictions describe only the release to hydrochloric acid 0.07 mol/l. Nevertheless recovery rates for the added amines in the collaborative trial matrix are displayed in table 5. They vary from 19 to 90% (2,4-diaminoanisole not taken into consideration).

In addition the recovery rates using a standard solution of the amines are displayed. The mean values vary from 91 to 106% (2,4-diaminoanisole not taken into consideration).

Note: Application of the additional cleaning step (not used for the collaborative trial) to a standard solution reduces the recovery rates for 2,4-diaminoanisole and 2,4-diaminotoluene to approximately 10 to 30%. For all other amines, only a minor decrease can be observed.

Discussion

The present method can be used for the detection and quantification of small amounts of primary aromatic amines, which can be released from water-colours

Table 5. Collaborative trial, recovery rates from sample 2 and standards

Compound	added amount mg/kg	sample 2 recovery % (\bar{x} lab. 1-4)	standard recovery % (lab. 1, $n = 3$)	standard recovery % (lab. 2, $n = 1$)	standard recovery % (lab. 3, $n = 3$)	standard recovery % (lab. 4, $n = 3$)
2,4-diaminoanisole	3.05	<i>n.a.</i>	93 ($s=2$)	<i>n.d.</i>	76 ($n=1$)	70 ($s=16$)
2,4-diaminotoluene	11.88	32	98 (2)	87	96 ($s=2$)	94 (4)
aniline	15.10	88	94 (1)	100	93 (2)	94 (1)
benzidine	4.66	71	106 (5)	95	109 (3)	101 (4)
p-toluidine	3.84	90	92 (1)	93	96 (2)	92 (2)
o-dianisidine	9.58	55	84 (12)	83	85 (5)	93 (3)
o-tolidine	6.58	68	93 (8)	90	110 (3)	93 (3)
1-naphthylamine	0.00	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>
2-naphthylamine	4.90	72	97 (1)	91	109 (1)	99 (2)
4-aminobiphenyl	12.00	81	97 (1)	94	110 (2)	98 (2)
3,3'-dichlorbenzidine	19.65	19	81 (3)	87	144 (9)	89 (2)
\bar{x}		64	94	91	106	95
Σ	88.19	57				

n.d. = not determined, *n.a.* = not applicable, lab. = laboratory, *italics: not used for calculation in this table*, further explanations: see text

under physiological conditions (hydrochloric acid 0.07 mol/l as gastric juice simulant, 37 °C). Detection limits of the analysed amines are 6 to 50 times below the legal limit of release (5 mg/kg). Quantitation limits as defined above are between 1 to 5 mg/kg.

The results of the collaborative trial confirm that the Swiss legal restrictions for the mentioned amines can be supervised with this method, except for 2,4-diaminoanisole which can only be determined qualitatively. 2,4-diaminoanisole is a very unstable compound. It decomposes very quickly even as a standard solution and it is therefore unlikely to be stable in water colours. The data for 3,3-dichlorbenzidine and 2,4-diaminotoluene show bad respectively unsatisfactory reproducibility, probably because of the low recovery rates in the collaborative trial matrix. We therefore recommend to confirm data of 2,4-diaminotoluene and 3,3-dichlorbenzidine by multiple determination. The individual and the sum results of the other detected amines indicate a spread that was expected for this matrix and concentrations. For *o*-dianisidine and *o*-tolidine, the repeatability is higher than the reproducibility. This behaviour seems to be a problem of statistical interpretation considering the small number of participants. All laboratories could confirm, that sample 2 was spiked with 2-naphthylamine and not with 1-naphthylamine, therefore the selectivity of this method regarding the differentiation of these two compounds is proven.

Summary

In Switzerland legal limits exist concerning the release of primary aromatic amines from water-colours for children. The extraction has to take place under physiological conditions (hydrochloric acid 0.07 mol/l as gastric juice simulant, 37 °C) without reductive cleavage of the azo dyes. A HPLC-method was refined which now allows the determination of 11 of these compounds simultaneously with UV-detection after a cleanup step over Extrelut®. Results are confirmed with a DAD Detector. The suitability of the method was tested in a collaborative trial.

Zusammenfassung

In der Schweiz existieren Grenzwerte für die Abgabe von primären aromatischen Aminen aus Wasserfarben für Kinder. Vorgeschrieben ist dabei eine Extraktion unter physiologischen Bedingungen (Salzsäure 0,07 mol/l als Magensaftsimulans, 37 °C) ohne reduktive Spaltung der Azo-Farbstoffe. Es wurde eine HPLC-Methode weiterentwickelt für die Bestimmung von primären aromatischen Aminen aus dem Salzsäureextrakt nach Reinigung über Extrelut®. Neu können 11 dieser Verbindungen gleichzeitig bestimmt werden. Die Detektion und Bestätigung erfolgt mittels UV- und DAD-Detektor. Die Tauglichkeit der Methode wurde in einem Ringversuch überprüft.

Résumé

Des valeurs limites pour les amines aromatiques primaires libérées par des peintures à l'eau destinées aux enfants sont en vigueur en Suisse. L'extraction dans des conditions

physiologiques (acide chlorhydrique 0,07 mol/l comme simulant du suc gastrique, 37 °C) sans scission par réduction du groupe azo des colorants est imposée. Une méthode HPLC est décrite pour la détermination des amines aromatiques primaires cédées au simulant, après purification de l'extrait sur colonne Extrelut®. 11 amines peuvent être déterminées simultanément par détection dans l'UV et les résultats sont confirmés au moyen d'un détecteur à barrettes de diodes. Le bon fonctionnement de la méthode a été testé par un essai interlaboratoire.

Literature

1. Verordnung über Gebrauchsgegenstände (GebrV) vom 1. 3. 1995 (SR 817.04, Bundeskanzlei Bern).
2. *Beuggert, H., Etournaud, A., Gerber, R., Hegersweiler, P. et Störi, M.*: Méthodes provisoires pour le matériel de peinture et de dessin, dosage des amines aromatiques cancérogènes. Manuel suisse des denrées alimentaires (Swiss Food Manual), chapitre 50. Eidg. Drucksachen- und Materialzentrale, Bern 1990.
3. *Fishbein, L.*: Aromatic amines of major industrial importance: Use and occurrence. Environmental carcinogens, selected methods of analysis, volume 4 – Some aromatic amines and azo dyes in the general and industrial environment, 51–74. WHO International Agency for Research on Cancer, Lyon 1981.
4. *Chernozemsky, I.N. and Boyland, E.*: Carcinogenicity of aromatic amines and azo dyes and their role in the development of human cancer. Environmental carcinogens, selected methods of analysis, volume 4 – Some aromatic amines and azo dyes in the general and industrial environment, 3–12. WHO International Agency for Research on Cancer, Lyon 1981.
5. Deutsche Forschungsgemeinschaft, MAK- und BAT-Werte-Liste 1994. Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe Mitteilung 30. VCH, Weinheim 1994.
6. *Ramseier, C., Schüpbach, M. und Séquin, U.*: HPLC-Bestimmung organischer Verunreinigungen in synthetischen Lebensmittelfarbstoffen. Mitt. Gebiete Lebensm. Hyg. **78**, 401–406 (1987).
7. Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG: Nachweis der Verwendung verbotener Azofarbstoffe auf gefärbten textilen Bedarfsgegenständen (B 82.02, 2). Beuth Verlag, Berlin 1996.
8. *Bertola, A.*, CLARIANT AG, personal message.
9. *Walter, E., Anderegg, P., Knutti, R., Kohler, P., Lavanchy, P., Lischer, P., Luisier, J.-L., Potterat, M. und Streuli, H.*: Statistik und Ringversuche, Schweizerisches Lebensmittelbuch (Swiss Food Manual), Kapitel 60B. Eidg. Drucksachen- und Materialzentrale, Bern 1989.
10. *Funk, W., Dammann, V. und Donnevert, G.*: Qualitätssicherung in der analytischen Chemie, XXIV. VCH, Weinheim 1992.

Dr. Christoph Bürgi
Kantonales Laboratorium
Basel-Stadt
Postfach
CH-4012 Basel