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

Herausgeber: Bundesamt für Gesundheit

Band: 89 (1998)

Heft: 2

Artikel: HPLC method for the determination of aromatic amines released from inks of ballpoint and fiber-tip pens under physiological conditions

Autor: Bürgi, Christoph / Bollhalder, Rita / Hohl, Christopher

DOI: https://doi.org/10.5169/seals-983139

Nutzungsbedingungen

Die ETH-Bibliothek ist die Anbieterin der digitalisierten Zeitschriften auf E-Periodica. Sie besitzt keine Urheberrechte an den Zeitschriften und ist nicht verantwortlich für deren Inhalte. Die Rechte liegen in der Regel bei den Herausgebern beziehungsweise den externen Rechteinhabern. Das Veröffentlichen von Bildern in Print- und Online-Publikationen sowie auf Social Media-Kanälen oder Webseiten ist nur mit vorheriger Genehmigung der Rechteinhaber erlaubt. Mehr erfahren

Conditions d'utilisation

L'ETH Library est le fournisseur des revues numérisées. Elle ne détient aucun droit d'auteur sur les revues et n'est pas responsable de leur contenu. En règle générale, les droits sont détenus par les éditeurs ou les détenteurs de droits externes. La reproduction d'images dans des publications imprimées ou en ligne ainsi que sur des canaux de médias sociaux ou des sites web n'est autorisée qu'avec l'accord préalable des détenteurs des droits. En savoir plus

Terms of use

The ETH Library is the provider of the digitised journals. It does not own any copyrights to the journals and is not responsible for their content. The rights usually lie with the publishers or the external rights holders. Publishing images in print and online publications, as well as on social media channels or websites, is only permitted with the prior consent of the rights holders. Find out more

Download PDF: 16.03.2026

ETH-Bibliothek Zürich, E-Periodica, https://www.e-periodica.ch

Mitt. Gebiete Lebensm. Hyg. **89**, 177–187 (1998)
Eingegangen 2. Februar 1998. Angenommen 4. März 1998

HPLC Method for the Determination of Aromatic Amines Released from Inks of Ballpoint and Fiber-tip Pens under Physiological Conditions

Key words: Aromatic amines, HPLC, SPE, Ink, Ballpoint pen, Fiber-tip pen

Christoph Bürgi, Rita Bollhalder, Christopher Hohl, Urs Schlegel and *André Herrmann*
Kantonales Laboratorium Basel-Stadt, Basel

Introduction

Dyes are major components of inks used in ballpoint and fiber-tip pens. Azo dyes may be contaminated with free aromatic amines, some of which are harmful or even carcinogenic. When using these types of pens, children may incorporate amines via mouth or skin contact. In Switzerland legal restrictions limit the maximum release of aromatic amines from inks of pens designed for children (1). Two limits are defined depending on the carcinogenic potential of the amine. Extraction is required to be done under simulated physiological (gastric) conditions (1, 2). Because of the differing limits, amines must be determined individually requiring a chromatographic separation instead of colorimetric determination of the sum. In the case of water insoluble inks, components interfere with HPLC determination rendering our formerly described method for water-colours useless (2). To our knowledge no method concerning the determination of individual amines in inks has been published to this date. In this paper we would like to show that amines can be determined in inks of both pen types provided that sample preparation is done with suitable SPE columns.

Method

Materials and instruments

Water bath with magnetic stirrer and immersion thermostat (e.g. IKA-RTM «basic» complete with five stirring points), Isolute® columns C2 2 g/12 ml ict AG Basel IST 320-0200-D, Isolute® columns C8 2 g/12 ml ict Basel IST 290-0200-D, filter 0.45 µm (e.g. Nylon Acrodisc 13, SKAN AG, 4009 Basel, prod. Nr. 4426), polyethylene syringes 1 ml

HPLC-system : Waters 600 MS pump, Waters 484 MS detector, Waters Model Code 600 column oven, ERMA tokyo ERC 3811 degasser, Millenium chromatography software, Waters 990 photodiode-array detector; column: LiChrosorb RP-18, 250 x 4 mm, 5 µm, precolumn 11 x 4 mm, Knauer GmbH, Berlin

GC/MS-system: Carlo Erba HRGC 5160, GCQ Finnigan Mat in EI mode; GC column: DB5-MS (J&W) 30 m x 0.25 mm I.D., d_f = 0,25 µm; Injector: on column

Reagents

Methanol p.a. (Merck 1.06009), trisodium phosphate-12-hydrate p.a. (Merck 1.06578), demin. water Nanopur R > 17 MΩ/cm^2, hydrochloric acid 25% p.a. (Merck 1.00316), water LiChrosolv (Merck 1.5333.2500), sodium dihydrogen phosphate monohydrate p.a. (Merck 1.06346), acetonitrile LiChrosolv (Merck 1.00030), ethylacetate AnalaR (BDH 101086J), sodium hydroxide p.a. (Merck 1.06545), diethyl ether p.a. (Merck 1.00921), toluene p.a. (Merck 1.08325), trifluoro acetic acid anhydride puriss. (TFAA) (Fluka 91719)

Reference materials

2,4-diaminoanisole (4-methoxy-1,3-phenylendiamine-sulfate-hydrate) 99.4% (Aldrich 18,332-6) CAS-Nr. 615-05-4, 2,4-diaminotoluene (2,4-toluylendiamine) pract. >98% (Fluka 33360) CAS-Nr. 95-80-7, aniline >99.5% (Merck 1.01261) CAS-Nr. 62-53-3, benzidine purum p.a. >99% (Fluka 121115) CAS-Nr. 92-87-5, o-toluidine purum p.a. (Fluka 89610) CAS-Nr. 95-53-4, m-toluidine puriss. p.a. >99% (Fluka 89620) CAS-Nr. 108-44-1, 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 >99% (Sigma D-9886) CAS-Nr. 91-94-1

Extractant (gastric juice simulant)

Dilute 9.8 g hydrochloric acid with demin. water to a total volume of 1000 ml resulting in a 0.07 M solution.

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. These solutions are stable for several months if stored at 4 °C in the dark, with the exception of 2,4-diaminosole which starts to decompose 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.

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 weighed-in quantity.

Eluant

Mix 662 g phosphate buffer pH 6 (dissolve 20.7 g sodium dihydrogenphosphate-monohydrate in approximately 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: Weighing buffer and acetonitrile gives best reproducibility.

HPLC parameters

Temperature: 40 °C, sensitivity: 0.020 aufs, flow rate: 1.00 ml/min, detection wave length: 17 min at 240 nm and 23 min at 278 nm, measuring time: 40 min, injection volume: 100 µl, DAD: Wavelength range: 200–400 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.

Sample preparation for ballpoint pens

Please check with figure 1. Pull cartridge out of the pen with a pair of tongs, pull off tip and discard follower if present. Place 2–3 cartridges (depending on cartridge size) in a 50 ml glass stoppered Erlenmeyer flask and let about 0.5 g ink drip out. Weigh ink immediately. If ink does not drip out on its own, push it out with a pasteur pipet or gently warm-up cartridge. Add exactly 50 times the amount of gastric juice simulant (ca. 25 ml) and homogenize in an ultrasonic bath for 5 min. Stir suspension under exclusion of light for 1 hour at 37 °C. After stirring, let sample stand for another hour at the same temperature and centrifuge. The raw extract can

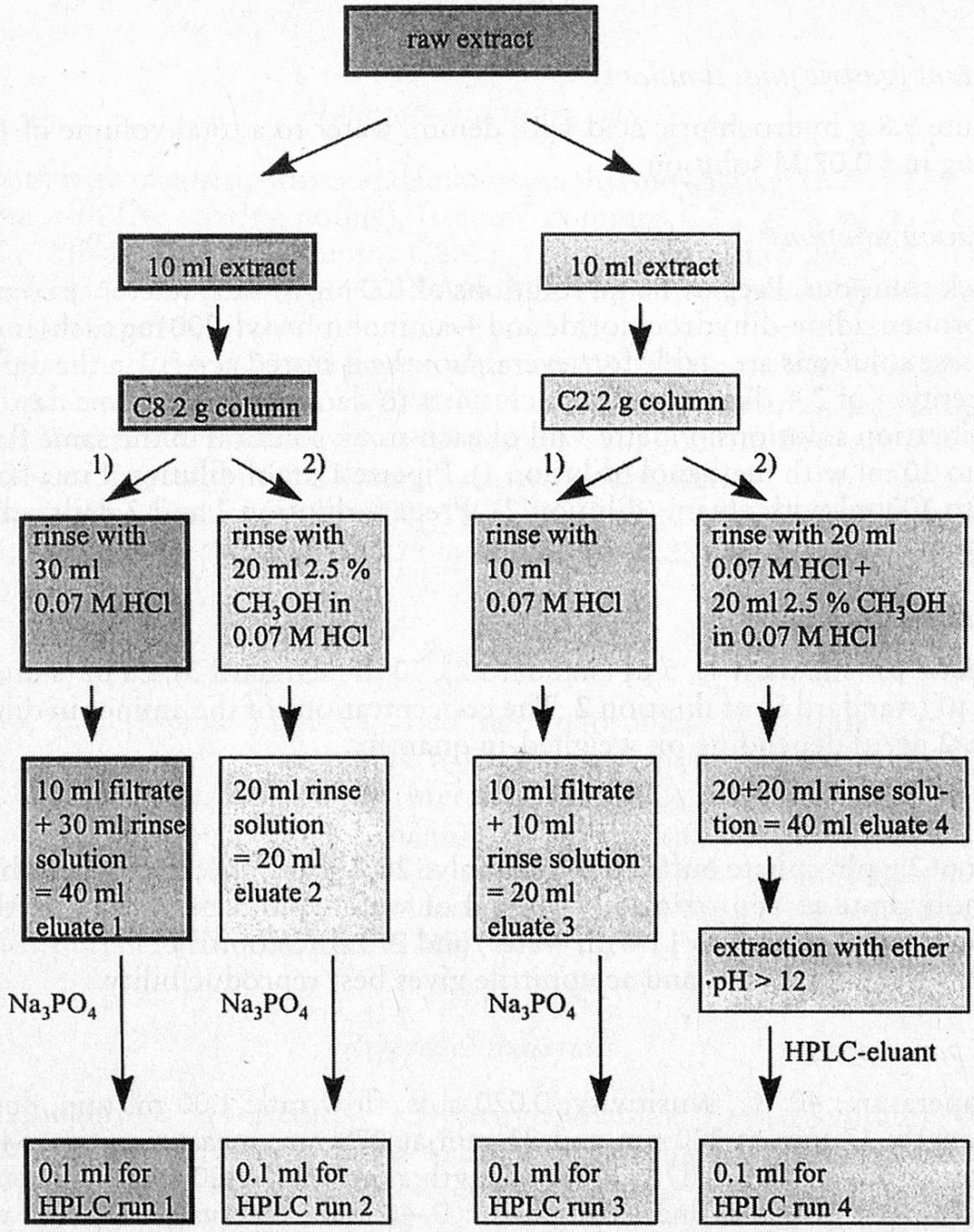

Fig. 1. Sample preparation for ballpoint pens. Routine analysis for aniline and o-toluidine consists of preparing eluate 1 only.

be stored for 12 hours under argon in a refrigerator. Condition a C2 and a C8 SPE cartridge with 10 ml methanol and then with 10 ml demin. water. Load each cartridge with 10 ml of extract and filter under vacuum (analytes are in the filtrate!). Rinse C8 column first with 30 ml of 0.07 M HCl (filtrate and rinse solution are *eluate 1*). Then rinse with 20 ml 2.5% methanol in 0.07 M HCl *(eluate 2).* Rinse C2 column first with 10 ml of 0.07 M HCl (filtrate and rinse solution are *eluate 3).* Then rinse column with 20 ml 0.07 M HCl and 20 ml 2.5% methanol in 0.07 M HCl (both rinse solutions are *eluate 4).* Adjust pH of *eluate 4* to 12 with 0.75 ml 5 M

NaOH and extract with 3 x 10 ml ether. Combine ether extracts, adjust to pH 1.5 with 2 ml 0.2 M HCl and evaporate only ether phase. Add HPLC eluant to an endvolume of 5 ml.

Adjust pH of the eluates 1–3 with trisodium phosphate-12-hydrate (532 mg for eluate 1, 266 mg each for eluate 2 and 3).

Remove residues from all four solutions with a nylon filter prior to injecting into the HPLC.

Sample preparation for water soluble fiber tip pens

Press liquid out of the cartridge with a tube squeezer (same device as used to press out mustard tubes). Weigh 0.5 g immediately in a 50 ml glass stoppered Erlenmeyer flask and add exactly 50 times the amount of gastric juice simulant (ca. 25 ml). Homogenize and extract as described above for ballpoint pens. Perform sample clean up as previously described for water-colours (2).

Note: Water soluble inks can be distinguished from insoluble inks by their higher dry weight (>10%) and indications on the package such as «washable at 40 °C».

Sample preparation for water insoluble fiber tip pens

Prepare raw extract as described above. Condition a C2 SPE cartridge with 10 ml methanol and then with 10 ml demin. water. Load cartridge with 20 ml extract and filter under vacuum (analytes are in the filtrate!). Rinse column twice with 20 ml 0.07 M HCl. Combine filtrate and rinse solutions and adjust pH to 6 with 798 mg trisodium phosphate-12-hydrate.

Sample preparation for semi-quantitative verification of aniline and o-toluidine in ball point pens with GC/MS

Extract sample as described above without further clean up. Adjust pH to > 12 with 5 M NaOH. Shake an aliquot with 2 x 5 ml of toluene, combine and evaporate, then adjust to a defined small end volume with toluene. Do not evaporate to dryness! Choose aliquot size and end volume according to the expected concentrations. Spike a second extract of the same sample with the expected amounts of amines. Dry both solutions with Na_2SO_4 add 1 drop of TFAA and shake well. The areas of amine peaks in the two extracts should differ by a factor of about 2.

Note: Glassware must be pretreated with toluene containing 1 drop of TFAA.

Parameters for GC/MS analysis

Temperature program: 90 °C, 1 min with 10 °C/min up to 100 °C, then with 2 °C/min up to 110 °C, then with 30 °C/min up to 200 °C, 5 min isothermal. Ionization mode: EI, sec per scan: 0.5, mass range: 50–210 am. He pressure: 60 kPa.

Determination of dry weight

Weigh approximately 200 to 500 mg of fresh ink. Dry for exactly 2 hours at 105 °C (3) and weigh again after cooling.

Results and Discussion

Clean up

While developing sample preparation, we soon realized that it would be too demanding a task to determine all amines of interest with one preparation procedure and a single HPLC run. Different types of ink contain components (4) which differ in their physico-chemical properties and elution behaviour on SPE columns. Moreover, in the case of ballpoint pen inks, the amines investigated had to be divided into two classes according to their retention properties during solid phase extraction. *Class A* comprised the amines with somewhat higher polarity (2,4-diaminoanisole, 2,4-diaminotoluene, aniline, benzidine, o-,m- and p-toluidine, o-dianisidine and o-tolidine), while the amines of *class B* were less polar (1- and 2-naphthylamine, 4-aminobiphenyl, 3,3'dichlorobenzidine). The optimal SPE columns were evaluated with spiked raw extracts in screening tests. For ballpoint pens, C8 cartridges proved to be the best medium for separating class A amines from interfering components in an acidic solution. Less retentive C2 cartridges on the other hand gave interference free chromatograms for class B amines but weren't capable of holding back interfering compounds relevant for class A, making the use of two SPE columns necessary. In addition to these separate pathways, quantitatively satisfactory results were only obtained, when each column was rinsed with a second eluate containing a small amount of methanol. This portion of organic solvent was just big enough to desorb amines adsorbed by the column without eluting interfering compounds. In addition to the different types of columns and eluates used, a concentration step of eluate 4 involving shaking with ether had to be added in order to improve detection limits. Hence checking 13 amines in ballpoint pen inks became a rather lengthy task!

Recovery

The recovery rates of 10 amines in standard solutions which were worked up as a ballpoint pen extract are shown in table 1. Recovery rates are above 80% with the exception of dichlorobenzidine and diaminoanisole. The necessity of rinsing each column with 2 eluates is also shown: of the class A amines about 39% of o-dianisidine and 60% of the recovered o-tolidine are found in eluate 2. In the case of class B amines, even larger portions of the recovered analytes were found in eluate 4: 67% of 2-naphthylamine, 100% 4-aminobiphenyl and 89% dichlorobenzidine. Recovery rates of the class A amines in a spiked ballpoint pen extract were greater

Table 1. Recovery rates and distribution of amines

	Standard Solutions*						Spiked ballpoint pen extract*	
	C8 column		C2 column					
	eluate 1	eluate 2	eluate 3	eluate 4	sum	s**	sum	s**
diaminoanisole	34.5%	4.5%	–	–	39.0%	30.6	14.8%	2.2
diaminotoluene	88.6%	3.3%	–	–	91.9%	4.5	76.0%	13.2
aniline	91.3%	0.0%	–	–	91.3%	2.9	113.4%	19.1
benzidine	100.4%	1.0%	–	–	101.4%	1.8	95.0%	9.4
o-toluidine	90.4%	0.0%	–	–	90.4%	2.4	92.9%	4.1
o-dianisidine	63.5%	35.5%	–	–	99.0%	5	75.3%	10.1
o-tolidine	36.5%	55.1%	–	–	91.6%	8.3	83.2%	7.8
2-naphthylamine	–	–	28.5%	57.5%	86.0%	1.9	60.0%	18.4
4-aminobiphenyl	–	–	0.0%	81.0%	81.0%	3.3	59.9%	21.8
dichlorobenzodine	–	–	6.6%	53.7%	60.3%	3.2	25.4%	6.6

* average of 3 solutions
** standard deviation of sum

than 75% except for 2,4-diaminosole. Class B amines on the other hand show significantly lower rates in comparison to the recovery rates found for standard solutions. The losses of class B amines could be explained by their reaction with matrix components e.g. the formation of adducts. These are however not of significance because the scope of this work is limited to free extractable amines. The quality of recovery can therefore be judged on the basis of standard solutions alone. Simplifying the procedure by combining eluates 1 and 2 respectively 3 and 4 is not possible because of matrix effects. Detection limits for HPLC are given in (2).

Applications

The diode array detector is mainly used to distinguish amines from interfering compounds (e.g. aniline from the solvent phenoxyethanol) and to determine peak purity. It is also useful in distinguishing 1- and 2-naphthylamine, which have the same retention time but differing carcinogenity (5, 6). A reliable differentiation of the more carcinogenic o-toluidine from the m- and p-isomeres (5–7) can only be done with GC retention times and the mass spectra of the derivatives. In order to exclude erroneous results, positive findings should be confirmed with GC/MS because separation and detection are based on totaly different principles than HPLC.

The practicability of the method was then tested in a market survey comprising 24 water soluble fiber-tip pens and 26 ballpoint pens. Whereas no amines were detected in fiber-tip pens, 10 ballpoint pens contained extractable aniline and o-toluidine in concentrations ranging between 56 and 1400 mg/kg respectively between 16 and 98 mg/kg (fig. 2a and fig. 2b). Of the 13 amines of interest, these two compounds were the only amines detected (fig. 3a and fig. 3b). DAD and GC/MS

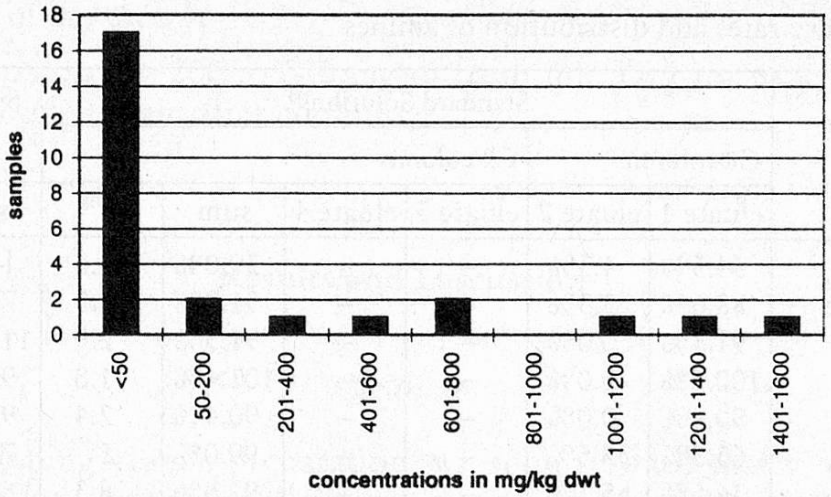

Fig. 2a. Concentrations of aniline in ballpoint pens

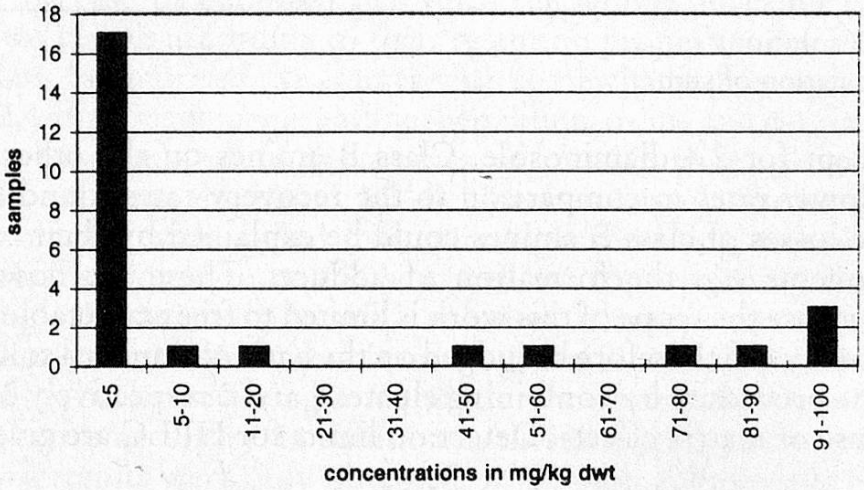

Fig. 2b. Concentrations of o-toluidine in ballpoint pens

analysis did however show evidence that some alkylated aniline compounds were also to be found, but these findings were not further regarded in this study. From the analytical aspect the effort for the routine determination of aniline and o-toluidine in inks can be drastically reduced because sample clean up only consists of making eluate 1 (fig. 1).

In judging a possible health hazard for children, one must take into account the carcinogenic potential which is considered to be greater for o-toluidine than it is for aniline (5, 6). The Swiss limit for definitely carcinogenic amines in inks of toy pens is 5 mg/kg dry weight respectively 50 mg/kg for suspected carcinogens. The first limit would apply to o-toluidine, the second to aniline. Both can penetrate human skin (6). Of the five toy pens analysed, one contained o-toluidine in concentrations 8 times above the limit. In this case law enforcement is based on a simple application of the limit. In the case of normal pens one has to take into consideration that children also use these pens (especially the cheap ones) and that concentrations found in these products were many times above the limits. We also

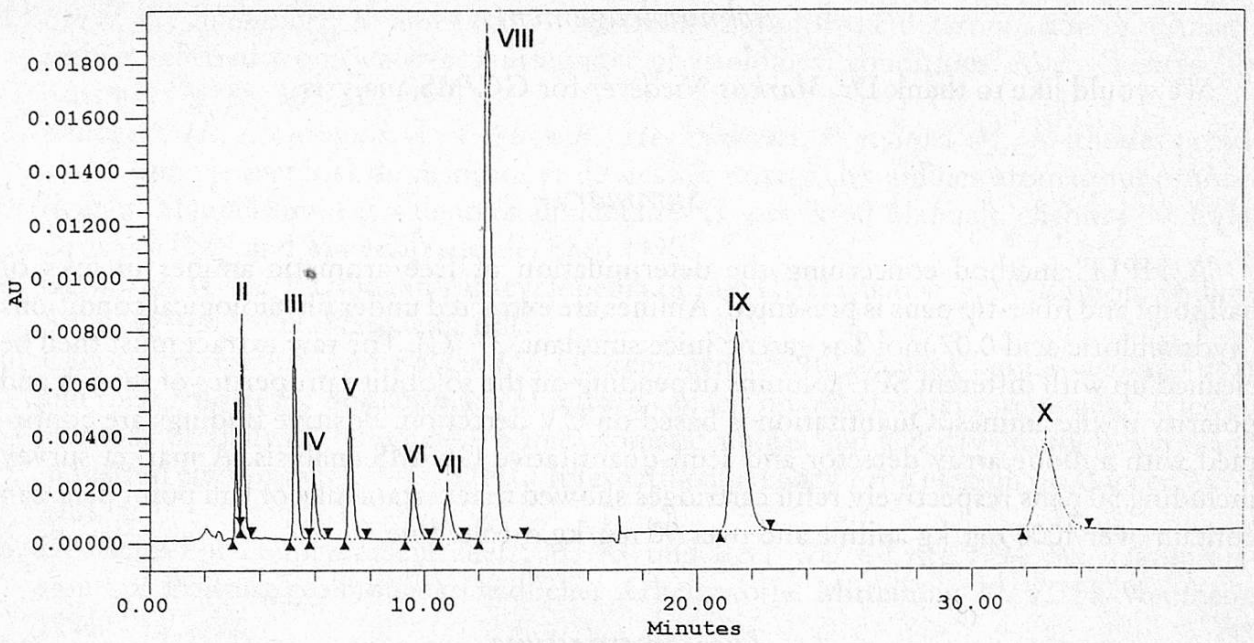

Fig. 3a. HPLC run of amine standards: I = diaminosole, II = diaminotoluene, III = aniline, IV = benzidine, V = o-toluidine, VI = o-dianisidine, VII = o-tolidine, VIII = 2-naphthylamine, IX = 4-aminobiphenyl, X = dichlorobenzidine (conditions see procedures)

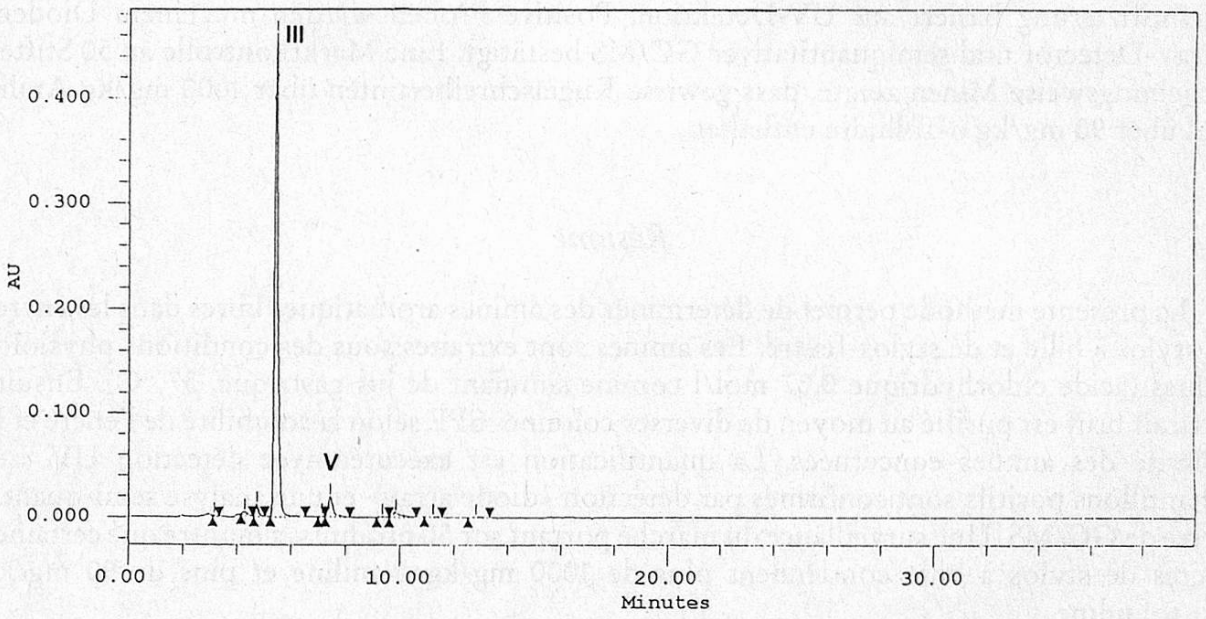

Fig. 3b. HPLC run of eluate 1 of a ballpoint pen containing aniline (III) and o-toluidine (V)

know that inks can be produced without relevant amounts of amines. We therefore advocate lowering this possible health hazard by using clean inks in the production of pens.

Acknowledgements

We would like to thank Dr. *Markus Niederer* for GC/MS analysis.

Summary

A HPLC method concerning the determination of free aromatic amines in inks of ballpoint and fiber-tip pens is presented. Amines are extracted under physiological conditions (hydrochloric acid 0.07 mol/l as gastric juice simulant, 37 °C). The raw extract must then be cleaned up with different SPE columns depending on the solubility properties of the ink and polarity of the amines. Quantitation is based on UV detection. Positive findings are confirmed with a diode array detector and semi-quantitative GC/MS analysis. A market survey including 50 pens respectively refill cartridges showed that certain inks of ball point pens can contain over 1000 mg/kg aniline and over 90 mg/kg o-toluidine.

Zusammenfassung

Es wird eine HPLC-Methode beschrieben, mit der freie aromatische Amine in Kugelschreibertinten sowie in Filzstift- und Faserstiftflüssigkeiten bestimmt werden können. Die Amine werden unter physiologischen Bedingungen extrahiert (Salzsäure 0,07 mol/l als Magensaftsimulans, 37 °C). Anschliessend wird der Rohextrakt je nach Löslichkeitseigenschaften der Tinte und Polarität der Amine mit verschiedenen SPE-Säulen gereinigt. Die Quantifizierung basiert auf UV-Detektion. Positive Proben werden mit einem Dioden-Array-Detector und semiquantitativer GC/MS bestätigt. Eine Marktkontrolle an 50 Stiften beziehungsweise Minen zeigte, dass gewisse Kugelschreibertinten über 1000 mg/kg Anilin und über 90 mg/kg o-Toluidin enthalten.

Résumé

La présente méthode permet de déterminer des amines aromatiques libres dans les encres de stylos à bille et de stylos-feutre. Les amines sont extraites sous des conditions physiologiques (acide chlorhydrique 0,07 mol/l comme simulant de jus gastrique, 37 °C). Ensuite l'extrait brut est purifié au moyen de diverses colonnes SPE selon la solubilité de l'encre et la polarité des amines concernées. La quantification est exécutée avec détection UV. Les échantillons positifs sont confirmés par détection «diode array» et une analyse semi-quantitative de GC/MS. Une surveillance du marché portant sur 50 produits, a montré que certaines encres de stylos à bille contiennent plus de 1000 mg/kg d'aniline et plus de 90 mg/kg d'o-toluidine.

Literature

1. Verordnung über Gebrauchsgegenstände (GebrV) vom 1.3.1995 (SR 817.04, Bundeskanzlei Bern).

2. *Bürgi, C., Bollhalder, R.* and *Otz, T.:* HPLC method for the determination of aromatic amines released from water-colours under physiological conditions. Mitt. Gebiete Lebensm. Hyg. **88**, 305–320 (1997).
3. *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 cancérigènes. Manuel suisse des denrées alimentaires (Swiss Food Manual), chapitre 50. Eidg. Drucksachen- und Materialzentrale, Bern 1990.
4. *Gerhartz, W. (ed.):* Ullmann's Encyclopedia of industrial chemistry, 5th Edition, volume A9, p. 42–45. VCH, Weinheim 1985.
5. *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.
6. Deutsche Forschungsgemeinschaft, MAK- und BAT-Werte-Liste 1994. Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe, Mitteilung 30, VCH, Weinheim 1994.
7. Toxicity indications in the Fluka catalogue. Fluka AG, Buchs 1997.

Kantonales Laboratorium BS
Dr. Christopher Hohl
Postfach
CH-4012 Basel