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Short communication

Formation of Schiff bases with acetone as a solvent in the determination of anilines

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Abstract

The formation of Schiff bases, when using acetone as solvent in the determination of aniline and substituted basic anilines, was verified by GC–MS and GC–Fourier transform measurements. These reaction products are detected as additional peaks in GC, impairing calibration and quantification.

1. Introduction

Several papers have been published dealing with direct GC procedures for the determination of polar organic compounds such as chlorinated anilines and phenols [1–4]. Such polar compounds are determined utilizing certain capillary columns without being derivatized.

In residue analyses, acetone is a widely used solvent for extraction and clean-up procedures in analytical chemistry, because of its volatility and wide ability to dissolve different compounds, and for mixing with other solvents. Sometimes it is also used to dissolve polar reference substances. However, its volatility and effect as a sensitizer for photochemical reactions [5] can impair analytical results. We have detected two peaks in gas chromatograms of 4-chloroaniline standards that were dissolved in hexane–acetone mixtures. The peak ratio changed over several days, preventing

reliable calibration and quantification. This suggested a reaction of 4-chloroaniline. Böer et al. [2] also reported additional peaks in gas chromatograms of acetone eluates of substituted anilines after solid-phase extraction on RP-18 material. They concluded that the formation of Schiff bases (ketimines) was responsible for these peaks. However, other workers have utilized acetone as a solvent for substituted anilines, which are used as internal standards [6], or as an eluting solvent combined with methylene chloride in adsorption chromatography on silica gel as part of an analytical method for priority pollutants including anilines, without reporting additional peaks [7]. The preparative synthesis of ketimines with various anilines (aniline, 4-chloroaniline, 4-bromoaniline, etc.) and acetone at room temperature using a dehydrating agent has been reported [8]. In this work, various substituted anilines were investigated to establish whether they react in standard solutions with acetone to Schiff bases, as postulated.

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2. Experimental

2.1. Chemicals

Acetone and hexane were obtained from Baker (Deventer, Netherlands), diethyl ether from Promochem (Wesel, Germany) and aniline, 4-isopropylaniline (4-IPA), 2-chloroaniline (2-CA), 3-chloroaniline (3-CA), 4-chloroaniline (4-CA), 2,4-dichloroaniline (2,4-DCA), 3,4-dichloroaniline (3,4-DCA), 2,4,5-trichloroaniline (2,4,5-TCA), 4-bromoaniline (4-BA) and ben-zidine from Amchro (New Haven, CT, USA).

2.2. Preparation of standard solutions

Standard solutions of the investigated anilines were prepared at a concentration of $1 \mu\text{g}/\mu\text{l}$ by dissolving 10 mg of the aniline in 1 ml of diethyl-ether and diluting to 10 ml with hexane.

2.3. Gas chromatography with flame ionization (GC-FID) and nitrogen-phosphorus detection (GC-NPD)

An HP 5890 Series II gas chromatograph equipped with an HP 7673 autosampler (Hewlett-Packard, Avondale, PA, USA), a flame ionization detector and a DB-5 fused-silica capillary column ($30 \text{ m} \times 0.25 \text{ mm}$ I.D.; $0.25 \mu\text{m}$ film thickness) (J&W Scientific, Folsom, CA, USA) was employed. The carrier gas was helium at a flow of 1 ml/min. The column temperature was programmed from 60°C (1 min isothermal) to 190°C at $8^\circ\text{C}/\text{min}$ and then to 260°C at $10^\circ\text{C}/\text{min}$. Injection was performed in the splitless mode at an injector temperature of 260°C . The detector was operated at 280°C with hydrogen at 30 ml/min and synthetic air at 350 ml/min as burning gases and nitrogen at 30 ml/min as make-up gas. A second HP 5890 Series II gas chromatograph was equipped with a nitrogen-phosphorus detector and a Stabilwax-DB fused-silica capillary column ($15 \text{ m} \times 0.25 \text{ mm}$ I.D.; $0.1 \mu\text{m}$ film thickness) (Restek, Bellefonte, PA, USA). Helium was used as the carrier gas at a flow-rate of 1.2 ml/min. The column tempera-

ture was programmed from 100°C (1 min isothermal) at $5^\circ\text{C}/\text{min}$ to 200°C , held for 5 min, and then at $25^\circ\text{C}/\text{min}$ to 230°C , held for 5 min. Injection was performed manually in the splitless mode. The injector temperature was 250°C . The detector was operated at 250°C with hydrogen at 3 ml/min and synthetic air at 100 ml/min as burning gases and nitrogen at 30 ml/min as make-up gas.

2.4. Gas chromatography-mass spectrometry (GC-MS)

An HP 5890 A Series II gas chromatograph was interfaced to an HP 5971 A mass-selective detector. The gas chromatograph was equipped with an HP 7673 autosampler and an Ultra-1 fused-silica capillary column ($25 \text{ m} \times 0.2 \text{ mm}$ I.D.; $0.33 \mu\text{m}$ film thickness) (Hewlett-Packard). Helium was used as the carrier gas at a flow-rate of 0.8 ml/min. The temperature programme was the same as in GC-FID. Injection was performed in the splitless mode at an injector temperature of 260°C . The temperature of the transfer line was 280°C and the temperature of the mass spectrometer was 185°C . The mass spectrometer was operated in the electron impact mode at 70 eV and in the scan range 50–500 u. The multiplier voltage was 1.75 kV.

2.5. Gas chromatography-Fourier transform infrared spectrometry (GC-FT-IR)

GC-FT-IR was performed on an HRGC 5160 gas chromatograph (Carlo Erba, Milan, Italy), which was interfaced to a model 740 FT-IR spectrometer (Nicolet, Madison, WI, USA). The gas chromatograph was equipped with a DB-1 fused-silica capillary column ($30 \text{ m} \times 0.25 \text{ mm}$ I.D., $0.32 \mu\text{m}$ film thickness) (J & W Scientific). The carrier gas was helium at a head pressure of 150 kPa and the temperature programme was the same as in GC-FID. Injection was performed manually in the splitless mode at an injector temperature of 260°C . The transfer line was at 200°C and the light pipe at 200°C .

2.6. Procedures

A

About 15 ml of hexane were placed in 20-ml graduated flasks, then 200 μ l of aniline standard solution were added to each flask, followed by 200 μ l of acetone after mixing. The flask was filled up to 20 ml with hexane and shaken immediately. The mixtures were kept at 22°C. After 1, 2, 4 or 6 h, depending on the reaction rate, 0.5 ml of each mixture was transferred into GC vials and analysed by GC-FID. Further, part of each mixture was transferred into vials and analysed periodically, first at intervals of about 4 h and later at longer intervals of 8–24 h. This procedure was repeated for a period of 7 days. Acetone–aniline mixtures that provided GC-FID traces with additional peaks were analysed by GC-EI-MS for identification of these peaks. To confirm the GC-MS results, the solutions were also analysed by GC-FT-IR.

B

A 70-ml volume of water was spiked with 200 μ l of either 4-CA or aniline standard solution. After adding 100 ml of acetone and 50 ml of hexane, the solutions were extracted on a horizontal shaker for 12 h (frequency 250 min^{-1}). The mixture was transferred into a separating funnel, mixed with 20 g of sodium-chloride and extracted with 30 ml of dichloromethane. The organic phase was separated, dried and evaporated and the residue was dissolved in 2 ml cyclohexane–ethyl acetate. These samples were analysed by GC-EI-MS.

3. Results and discussion

A 4-CA solution was analysed by GC-MS for the identification of the two peaks that were observed in GC-FID and GC-NPD (see Fig. 1). The mass spectra are presented in Fig. 2. The first peak gave a mass spectrum with a base peak of m/z 127 and an isotope peak of m/z 129. It could be assigned to chloroaniline. The molecular ion of the second peak was 40 u heavier than the molecular ion of 4-CA and showed the same

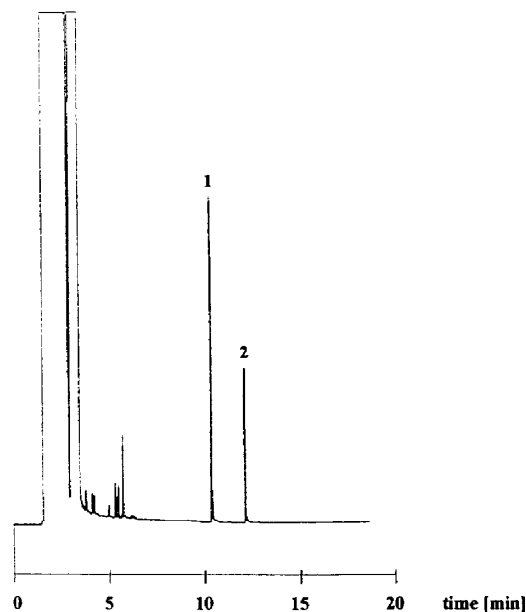


Fig. 1. GC-FID of a 4-CA solution (10 $\text{ng}/\mu\text{l}$) in hexane–acetone. Peaks: 1 = 4-CA; 2 = additional peak (Schiff base). Column, fused-silica coated with DB-5 bonded stationary phase (30 m \times 0.25 mm I.D., 0.25 μm film thickness); carrier gas, helium at 1.0 ml/min; temperature programme, from 60°C (1 min) at 8°C/min to 190°C and at 10°C/min to 260°C; injection, splitless (0.75 min) at 260°C.

chlorine cluster (m/z 167, 169). Additional information could be obtained from the fragmentations. The mass difference between the molecular and the fragment ion of 56 u pointed to the elimination of a $\text{C}_3\text{H}_6\text{N}$ fragment. The second fragment ion (m/z 152, 154) could be traced to the elimination of a CH_3 group. The detection of an additional peak using NPD confirmed that the compound contained nitrogen. From this information, the compound could be identified as Schiff base of 4-CA. The formation of the Schiff base could be verified by GC-FT-IR measurement. The IR spectrum of 4-CA exhibited two weak absorption bands with stretching vibrations at 3503 and 3411 cm^{-1} , which are characteristic of primary amines, and one band of medium intensity at 1623 cm^{-1} arising from the N–H deformation mode. These bands were absent in the spectrum of the second peak. Instead, a strong absorption band at 1667 cm^{-1} within the characteristic absorption region of the $-\text{N}=\text{C}$ -ketimine group appeared. GC-MS and GC-FT-

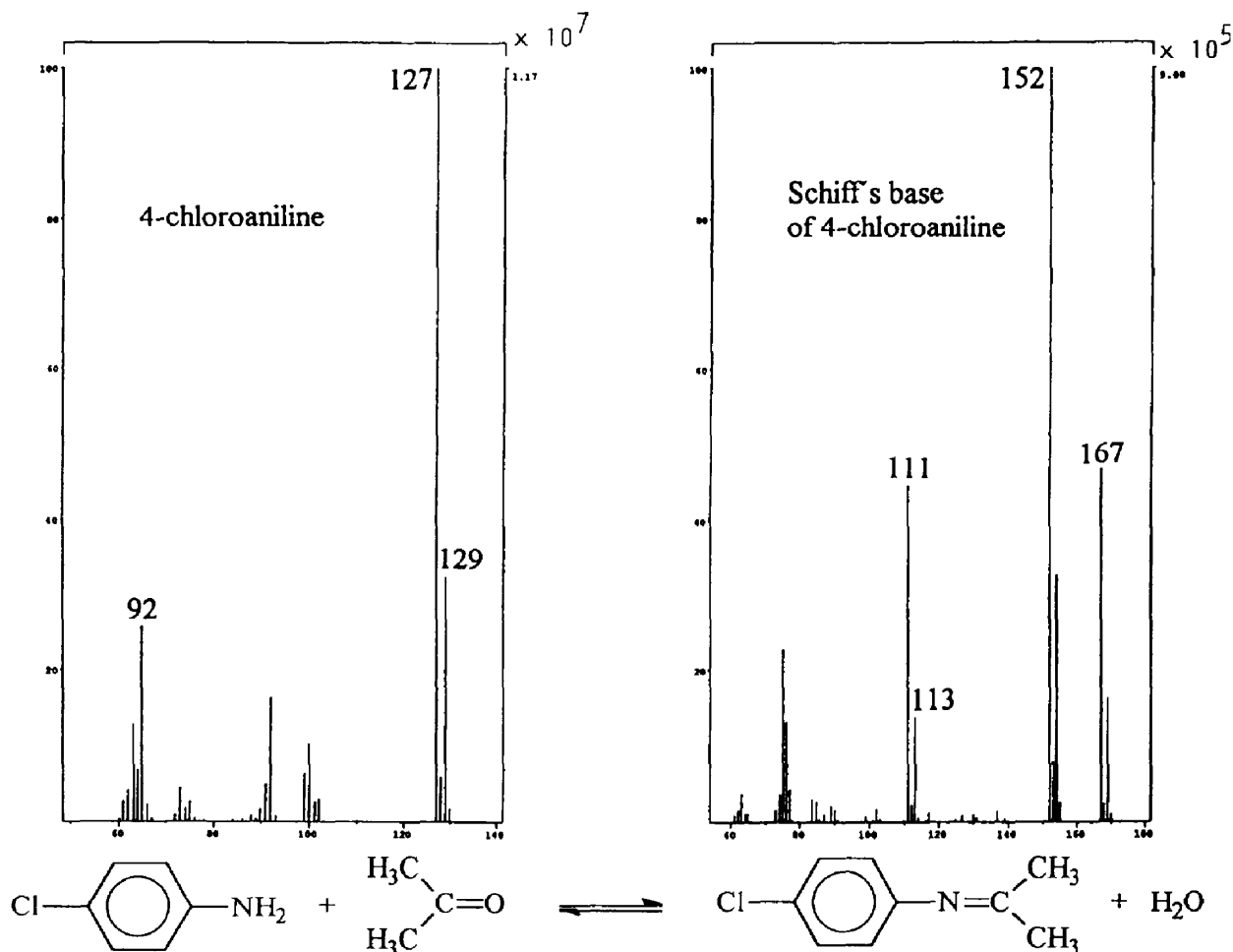


Fig. 2. EI mass spectra (scan range 50–500 u) of 4-CA (10 ng/ μ l) and the Schiff base of 4-CA formed in a solution containing acetone (1%, v/v). The reaction equation is also shown.

IR provided, in our opinion, the necessary evidence for the time-controlled formation of the Schiff base. It was also proved by Fig. 3, as will be discussed later.

The reaction extents and rates of formation of Schiff bases with further anilines were determined. After various reaction times, GC-FID of aniline, 4-IPA, 2-CA, 3-CA, 4-CA, 2,4-DCA and 4-BA mixtures with acetone showed an additional peak. The benzidine chromatogram even showed two additional peaks. No additional peak could be observed for the 2,4,5-TCA solution. By GC-MS measurement, all additional peaks could be identified as Schiff bases (N-

isopropylideneanilines), as the mass spectra of these peaks contained the characteristic mass difference of 40 u for the simultaneous formation of the ketimine group and elimination of water. The molecular and fragment ions of the anilines and their Schiff bases are listed in Table 1. The mass spectra of the reaction products of chloroanilines, bromoanilines and benzidine contained fragment ions with a mass difference of 56 u from the molecular ion masses, which is characteristic of the elimination of a C_3H_6N fragment. A further fragment ion of all ketimine mass spectra and the only in case of aniline and IPA ketimines resulted in elimination of a CH_3 frag-

Table 1

Molecular and fragment ions (GC–EI–MS) of anilines and their Schiff bases as reaction products with acetone (base peaks in italics)

Substance	Anilines (<i>m/z</i>)		Schiff bases with acetone (<i>m/z</i>)	
	Molecular ion	Fragment ion	Molecular ion	Fragment ion
Aniline	93	–	133	<i>118</i>
4-IPA	135	<i>120</i>	175	<i>160</i>
2-CA	<i>127/129</i>	–	167/169	<i>152/154, 111/113</i>
3-CA	<i>127/129</i>	–	167/169	<i>152/154, 111/113</i>
4-CA	<i>127/129</i>	–	167/169	<i>152/154, 111/113</i>
4-BA	<i>171/173</i>	92	211/213	<i>196/198, 155/157</i>
3,4-DCA	<i>161/163</i>	–	201/203	<i>186/188, 145/147</i>
2,4-DCA	<i>161/163</i>	–	201/203	<i>186/188, 145/147</i>
2,4,5-TCA	<i>195/197</i>	–	–	–
Benzidine	<i>184</i>	–	(a) 224 (b) 264	209, 168, 167 249, 152, 117

ment. The second product of the benzidine reaction was formed with two molecules of acetone (mass difference of another 40 u).

Fig. 3 shows the reaction rates and ratios of the different anilines. The ratios of the peak areas ($\text{area}_{\text{Schiff base}}/\text{area}_{\text{aniline}}$) depending on the reaction time are described here for selected anilines. As reference substances were unavailable, a quantification of the Schiff bases could not be performed. The order of the reaction ratios was 4-IPA > aniline > 4-CA > 3-CA > 4-BA > 3,4-DCA >> 2-CA > 2,4-DCA.

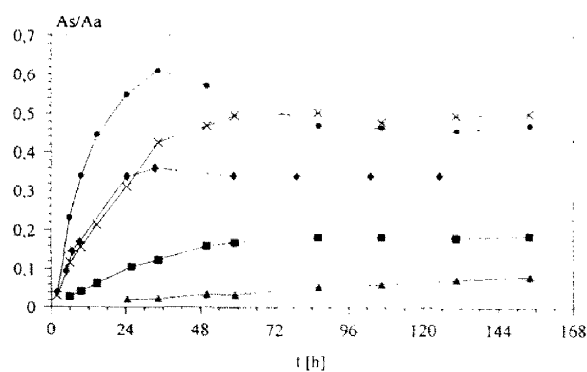


Fig. 3. Course of reaction for the formation of Schiff bases for selected anilines (10 ng/μl) and acetone (1%, v/v) in hexane at 22°C. Chromatographic conditions as in Fig. 1. As = peak area of Schiff base; Aa = peak area of aniline; *t* = time. ● = 4-IPA; × = aniline; ◆ = 4-CA; ■ = 3-CA; ▲ = 3,4-DCA.

After 5 and 15 days 2-CA and 2,4-DCA, respectively, showed a very small peak for the Schiff base. The order stated above is approximately related to the order of the basicity of the anilines and to the availability of the free electron pair at the nitrogen [9]. The first reaction product of benzidine was the ketimine with one molecule of acetone. The second ketimine with two molecules acetone was detected 6 h later.

Further, investigations were carried out to establish whether Schiff bases were formed from aniline and 4-CA during the common extraction of water with acetone–hexane, and whether they were stable during evaporation and dissolution procedures. By GC–MS the corresponding Schiff bases of both anilines were detected in the dissolved extracts despite the water content, which shifts the reaction balance to the disadvantage of Schiff base formation [10]. However, the peak areas of the Schiff bases were much smaller than with standard solutions, amounting to only 5% of the aniline peak areas.

The assumed formation of Schiff bases as reaction products of basic anilines and acetone could be verified at trace concentration levels. Therefore, aniline standards should not be dissolved using acetone. Acetone could be replaced by ethyl acetate, diethyl ether or other polar solvents. The formation of ketimines can cause problems during column chromatography when

using acetone as the eluent [2]. Further, small losses of anilines by formation of Schiff bases were observed during the extraction of spiked water with acetone.

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