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Liquid chromatography–mass spectrometry method for the determination of aldehydes derivatized by the Hantzsch reaction

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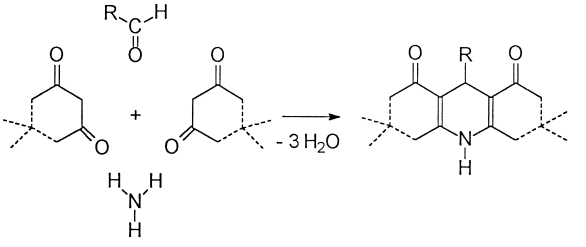
Abstract

A liquid chromatography–mass spectrometry method for the determination of aliphatic aldehydes after derivatization with acetylacetone or dimedone by means of the Hantzsch reaction is presented. Two molecules of a β-diketone, one molecule of ammonia and an aliphatic aldehyde cyclisate under formation of colored and fluorescent reaction products. Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) in the positive mode are suitable to ionize the formed dihydropyridine and decahydroacridine derivatives under protonation of their basic secondary amine functionality. The method has been used to identify the oxidation product of the formaldehyde derivatives as side product. The acetaldehyde derivative, presumably formed in the reaction of residual acetaldehyde in the acetic acid or acetate reagents, is mainly responsible for the increasing fluorescence background of the reagent solutions.

Keywords: Derivatization, LC; Hantzsch reaction; Aldehydes; Acetylacetone; Dimedone

1. Introduction

The derivatization of aldehydes, in most cases formaldehyde, by means of the Hantzsch reaction offers some interesting features compared to the more frequently used hydrazine reagents. The reaction is characterized by a cyclization of two β-dicarbonyl compounds and the aldehyde in the presence of ammonia to form a fluorescent heterocyclic system:



R= H, alkyl

The first β-diketone applied for this purpose under analytical aspects was acetylacetone [1], resulting in a dihydropyridine derivative. This is illustrated in the reaction scheme for the straight line structural formula. The use of 5,5-dimethyl-1,3-cyclohexanedione

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(dimedone) was first described by Sawicki and Carnes [2]. In this case, the derivatives are decahydroacridines, as shown for the dotted structural formula in the reaction scheme. The application of other β-dicarbonyl compounds as ethyl acetoacetate or cyclohexanedione is also described in Refs. [1,2]. The mechanism of the derivatization can be found in Ref. [3]. For liquid phase sampling, the derivatization requires elevated temperatures, e.g., 60°C [1,2] and reaction times between 30 min to 60 min to yield reasonable reaction rates.

Besides the direct fluorescence techniques, liquid chromatographic separation methods have been developed for both acetylacetone [4,5] and dimedone derivatives [6] to distinguish between different aldehydes.

Due to increasing background fluorescence in the reagent solutions, several publications describe laborious clean-up procedures for these, especially for cyclic diketones [6,7]. The target of this work was to elucidate the origin of the increasing blank of the reagent solution. Liquid chromatography–mass spectrometry (LC–MS) using ionization techniques at atmospheric pressure such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) [8] has established as a powerful tool for the identification of analytes and unknown interferents via their molecular mass. A high-performance liquid chromatography (HPLC)–MS method for the determination of aldehydes as acetylacetone and dimedone derivatives is developed in order to enhance the selectivity.

2. Experimental

2.1. Chemicals

All chemicals were purchased from Aldrich (Steinheim, Germany) in the highest quality available, with the following exceptions: as solvent for LC, acetonitrile gradient grade quality from Merck (Darmstadt, Germany) was used. Acids were also from Merck, 5,5-dimethyl-1,3-cyclohexanedione (dimedone) was from Fluka (Neu-Ulm, Germany).

2.2. Synthesis of the derivatives

The acetylacetone and dimedone derivatives of formaldehyde, acetaldehyde, propanal and butanal were synthesized according to the procedure stated in Refs. [1,2]. The identity of the products was confirmed by MS (electron ionization), ^{1}H nuclear magnetic resonance (NMR) spectrospcopy, IR spectroscopy, elemental analysis and comparison to the respective literature data [1–3].

2.3. HPLC–MS instrumentation

The HPLC–MS system from Shimadzu (Duisburg, Germany) consisted of the following components: a controller unit SCL-10Avp, a degasser DGU-14A, two pumps LC-10ADvp, a mixing chamber Model SUS (volume, 0.5 ml), an autosampler SIL-10A, a UV–Vis detector SPD-10A, a mass spectrometer LCMS QP8000 with atmospheric pressure ionization and software Class 8000 Version 1.11. For flow injection analysis (FIA)–MS and HPLC–MS experiments during method optimization, a manual injection valve Model 7520 from Rheodyne with fixed injection volume of 0.5 μl was used instead of the autosampler.

2.4. HPLC conditions

All separations with the HPLC–MS system were performed using a Merck LiChroSpher RP-18ec column with ChromCart cartridges (Macherey-Nagel, Düren, Germany) of the following dimensions: particle size, 5 μm; pore size, 100 Å; 125 mm×2 mm I.D.

2.4.1. A: Isocratic system

Separation of both acetylacetone and dimedone derivatives is achieved using a mixture of acetonitrile–water (40:60) containing 1% acetic acid as solvent at a flow-rate of 0.2 ml/min. The injection volume was 0.5 μl.

2.4.2. B: Binary gradient system for the separation of acetylacetone derivatives

An acetonitrile–water binary gradient at a flow-rate of 0.3 ml/min with the following profile was used:

Time (min)	0.01	1	8	12	13	16.5	17
c (CH_3CN) (%)	30	30	100	100	30	30	Stop

The injection volume was 5 μl. UV–Vis detection was carried out at detection wavelengths 251 and 365 nm.

2.4.3. C: Binary gradient system for the separation of dimedone derivatives

An acetonitrile–water binary gradient at a flow-rate of 0.3 ml/min with the following profile was used:

Time (min)	0.01	1	12	16	17	19.5	20
c (CH_3CN) (%)	30	30	100	100	30	30	Stop

Injection volume and detection wavelengths were the same as described for B.

2.5. MS conditions

The optimized MS conditions for the different derivatives and two types of ionization techniques are listed in Table 1. The curved desolvation line (CDL) is a charged and heated capillary responsible for the transmission of the ions into the mass spectrometer. The CDL is additionally the vacuum restrictor of the system. The detector gain was 1.5 kV for all measurements, the integration time was 1.5 s.

2.6. ESI- and APCI-MS (positive mode) spectra of acetylacetone and dimedone derivatives

The ESI- and APCI-MS spectra of the following acetylacetone and dimedone derivatives were recorded in the FIA–MS mode: formaldehyde, acetaldehyde, propanal, butanal. A 20-μl volume of each derivative solution in acetonitrile (10^{-4} mol/l) was injected into a stream of acetonitrile–water (49:51, v/v) at a flow-rate of 0.3 ml/min and analyzed in the scan mode.

2.7. Limits of detection (LODs) by means of HPLC–MS

Stock solutions of the acetylacetone and dimedone derivatives in acetonitrile (10^{-4} mol/l) were prepared and were stepwise diluted down to 10^{-6} mol/l for the acetylacetone derivatives and to $5 \cdot 10^{-8}$ mol/l for the dimedone derivatives. All solutions were analyzed each in triplicate by HPLC–MS in the single ion monitoring (SIM) mode using both ESI and APCI. The respective SIM traces are presented in Table 2.

3. Results and discussion

The derivatization of aldehydes based on the Hantzsch reaction is an interesting alternative to the commonly used hydrazine reagents, as only the derivatization products should exhibit fluorescent properties. All educts themselves in the reagent solution are non-fluorescent. The drawback of this method is, however that an increasing fluorescent background signal is observed in the reagent solution with time. In order to elucidate possible interferents from the reagent solution, a HPLC–MS method for the determination of acetylacetone and dimedone derivatives has been developed.

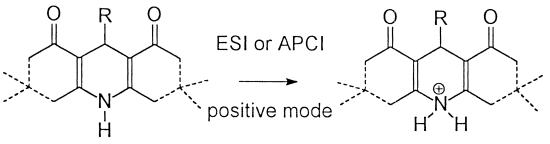
Table 1
Ionization parameters for acetylacetone and dimedone derivatives (CDL: curved desolvation line)

	Ionization technique	Probe voltage (kV)	APCI probe temperature (°C)	Nebulizer gas flow (N_2) (l/min)	CDL voltage (V)	CDL temperature (°C)	Deflector voltages (V)	Mass range (scan mode) (m/z)
Acetylacetone derivatives	ESI	4	–	4.5	−30	250	45	80–300
Acetylacetone derivatives	APCI	3.5	350	2	−30	280	55	80–300
Dimedone derivatives	ESI	4	–	4.5	−20	240	60	100–400
Dimedone derivatives	APCI	4	450	2	−20	280	55	100–400

Table 2
SIM traces for the $[M+H]^+$ of all derivatives and the respective limits of detection (LODs) for different ionization techniques

	Acetylacetone derivatives			Dimedone derivatives		
	m/z	LOD (mol/l), ESI	LOD (mol/l), APCI	*m/z*	LOD (mol/l), ESI	LOD (mol/l), APCI
Formaldehyde	194	10^{-5}	10^{-5}	274	$2\cdot10^{-6}$	$5\cdot10^{-6}$
Acetaldehyde	208	10^{-6}	10^{-6}	288	$2\cdot10^{-7}$	10^{-7}
Propanal	222	$5\cdot10^{-7}$	10^{-6}	302	10^{-7}	$2\cdot10^{-7}$
Butanal	236	$5\cdot10^{-7}$	$5\cdot10^{-7}$	316	$5\cdot10^{-8}$	10^{-7}

The class of dihydropyridines and decahydroacridines is characterized by the significant basicity of the secondary amino function. The ionization of these compounds at atmospheric pressure may therefore be carried out in the positive mode as presented in the scheme below. Both ESI and APCI are applied successfully:



R= H, alkyl

The ionization leads to pseudomolecular $[M+H]^+$ ions. As example, the APCI-MS spectrum of the dimedone derivative of acetaldehyde is shown in Fig. 1. The $[M+H]^+$ ion with *m/z* 288 is base peak, no further fragments except *m/z* 286 are detected. The peak at *m/z* 286 may be interpreted as $[M+H]^+$ of an oxidized species of the acetaldehyde derivative. The ESI- and APCI-MS spectra of all dimedone and acetylacetone derivatives have been recorded under the conditions described in Experimental. The $[M+H]^+$ ion is the dominant peak in any of the individual mass spectra. The respective pseudomolecular ions of all derivatives are provided in Table 2.

The separation of the derivatives is helpful for the unambiguous identification of the compounds. It is easily achieved using a reversed-phase HPLC column and acetonitrile–water as eluent. In Fig. 2, the isocratic separation of the dimedone derivatives of formaldehyde, acetaldehyde, propanal and butanal is

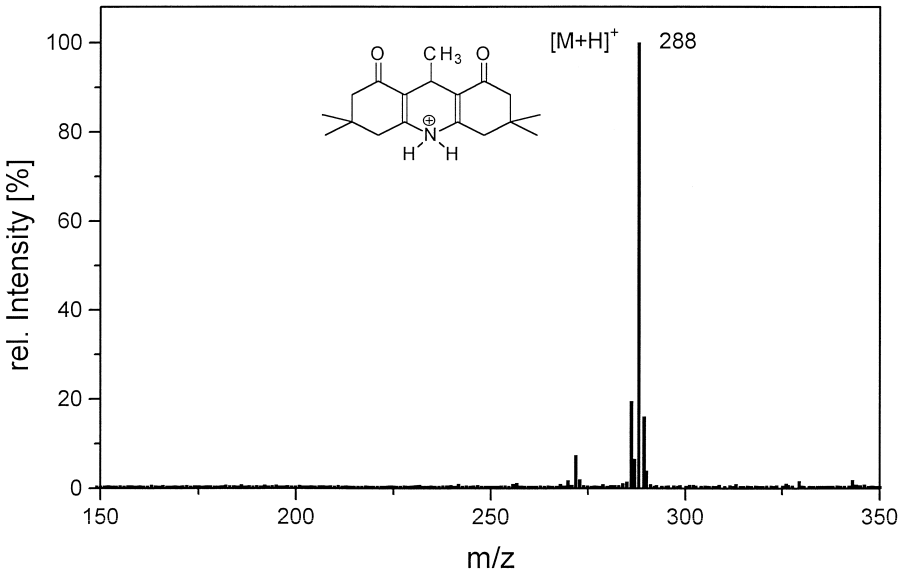


Fig. 1. APCI-MS spectrum of the acetaldehyde dimedone derivative.

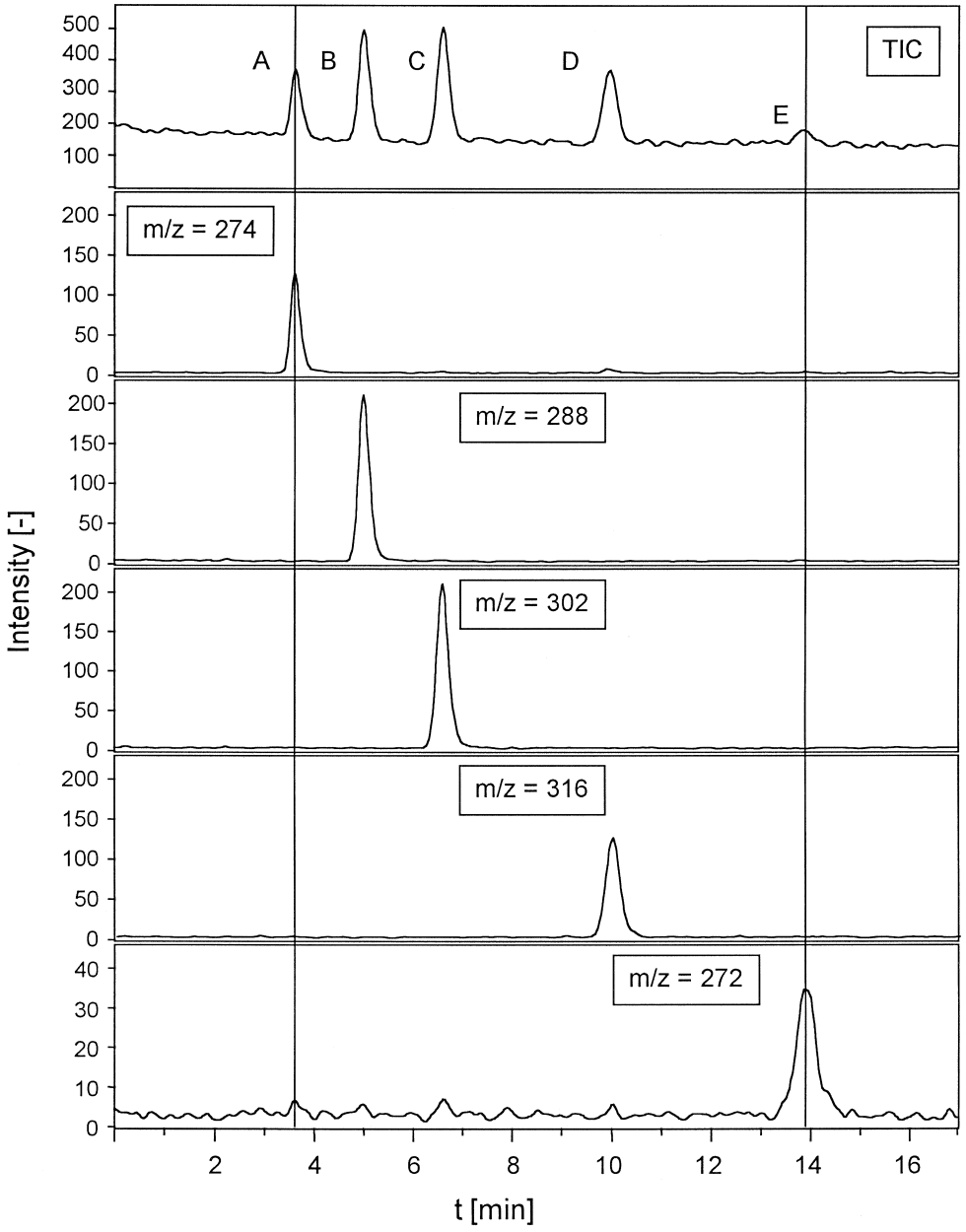


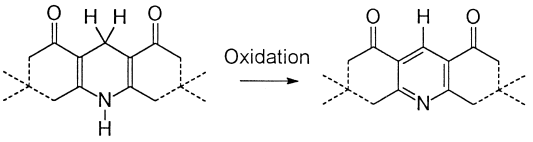
Fig. 2. Total ion current (TIC) chromatogram and extracted single ion monitoring traces of the dimedone derivatives of formaldehyde (A), acetaldehyde (B), propanal (C), butanal (D) and the oxidation product of A (E).

presented. The fifth peak (peak E) in the chromatogram may be assigned to the oxidation product of the formaldehyde derivative. This will be referred to later.

The separation may be further improved using a gradient elution system described in Experimental. The gradient elution has been applied for the determination of the instrumental LODs from standard solutions. The detection limits for the different ionization techniques are summarized in Table 2.

The LODs of the acetylacetone derivatives are up to 10-times higher than those of the dimedone derivatives. Using a single quadrupole mass spectrometer, the LOD is similar to the LOD of UV–Vis detection for both groups of derivatives and to the LOD of fluorescence detection in case of the acetylacetone derivatives, but higher compared to fluorescence detection of the dimedone derivatives. A further increase for the mass spectrometric determination of the derivatives appears to be possible with tandem mass spectrometric detection. A comparison of the LODs for one derivative group using different ionization techniques delivers similar results. Within one group of derivatives, the LODs improve with increasing alkyl chain length of the derivatized aldehyde.

The investigation of the standard solutions by HPLC–MS revealed an interesting fact: although only four solutions of solid standards were freshly prepared and mixed for analysis, a fifth peak eluting behind the butanal derivative of dimedone is observed (see Fig. 2). The ESI-MS spectra of the formaldehyde dimedone derivative (A) and the additional peak (E) show m/z 274 and 272, respectively, as base peaks. Compound E can therefore be considered as the oxidation product of the formaldehyde dimedone derivative according to the following equation:



This assumption has been proved by experiments of the formaldehyde derivative before and after storage. During storage of the formaldehyde derivative in solution, a continuous increase of m/z 272 is accompanied by a decrease of m/z 274. The dimedone derivatives of acetaldehyde, propanal and butanal are characterized by higher stability under storage conditions compared to the formaldehyde derivative. The respective oxidation products are only observed in the presence of strong oxidizing agents e.g., NO_x.

This instability of the formaldehyde derivative is also observed for its analogue derived from acetylacetone. The consequence of the instability is that no external calibration is possible for this compound. Additionally, this degradation explains the higher LODs of the formaldehyde derivatives.

A dimedone reagent solution prepared according to Mopper et al. [6] was stored at room temperature and daylight in order to fasten the aging process. The purpose of this analysis was to elucidate the origin of the increasing blank. Based on the peak formation in the HPLC with fluorescence detection, one major fluorescent interferent was suspected to be the dimedone derivative of acetaldehyde. This was finally confirmed by HPLC–ESI-MS. A possible source of acetaldehyde is the ammonium acetate or acetic acid in the reagent solution owing to the technical synthesis of acetic acid by oxidation of acetaldehyde [9]. The possibility for a determination of acetaldehyde with this method is therefore limited. The following consequences result from these measurements: (1) it is reasonable to prepare all reagent solutions as freshly as possible, (2) due to the fact that the formaldehyde derivatives are not stable and that the reaction is not quantitative, external calibration is not possible, and (3) all acetaldehyde measurements, with and without HPLC separation, should be evaluated critically due to the increasing acetaldehyde blank.

The most suitable approach is therefore the use of a flow system for the direct determination of formaldehyde as already described by Ref. [10]. In other cases, the calibration via the reaction is the only possibility to consider the low reaction rates and the non-quantitative reactions obtained.

The HPLC–MS method has therefore proved to be a valuable tool for the identification of the various products and side products resulting from the Hantzsch reaction with different reagents. The combination of liquid chromatography and mass spectrometry provides for extraordinarily high selectivity compared to the conventional detection approaches.

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References

[1] T. Nash, Biochemistry 55 (1953) 416–421.
[2] E. Sawicki, R.A. Carnes, Mikrochim. Acta, (1968) 148–159.
[3] B.J. Compton, W.C. Purdy, Can. J. Chem. 58 (1980) 2207.
[4] G. Chiavari, M.C. Faccini, S. Fuzzi, J. Chromatogr. 333 (1985) 262–268.
[5] M. Okamoto, K. Ohtsuka, J. Imai, F. Yamada, J. Chromatogr. 219 (1981) 175–178.
[6] K. Mopper, W.L. Stahovec, L. Johnson, J. Chromatogr. 256 (1983) 243–252.
[7] J. van Dijk, F. Boosma, G. Alberts, A.J. Man in 't Veld, M.A.D.H. Schalekamp, J. Chromatogr. B 663 (1995) 43–50.
[8] W.M.A. Niessen, A.P. Tinke, J. Chromatogr. A 703 (1995) 37–57.
[9] K. Peter, C. Vollhardt, in: Organische Chemie, VCH, Weinheim, 1988, p. 785.
[10] T.J. Kelly, C.R. Fortune, Int. J. Environ. Anal. Chem. 54 (1994) 249–263.