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Analysis of Pyrimidine Derivatives by Capillary Electrophoresis

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Summary. A capillary electrophoretic method has been developed for the determination of the main product as well as of by-products in technical samples of substituted pyrimidines. Both zone electrophoresis and micellar electrokinetic chromatography have been used for the separation employing electrolytes consisting of borate buffers (*pH* 9 to 9.4) with or without sodium dodecylsulfate. Optimization of separation selectivity could be achieved by addition of up to 20% 2-propanol or methanol to the carrier electrolyte. Quantification by internal standards resulted in relative standard deviations between 0.2 and 0.8%. By-products could be analyzed down to levels of 0.1% in technical samples.

Keywords. Capillary electrophoresis; Pyrimidines; Micellar electrokinetic chromatography; Organic solvents in capillary electrophoresis, Industrial product control.

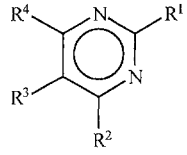
Analyse von Pyrimidinderivaten mitteles Kapillarelektrophorese

Zusammenfassung. Für die Bestimmung von Haupt- und Nebensubstanzen in technischen Proben von substituierten Pyrimidinen wurde ein kapillarelektrophoretisches Analysenverfahren entwickelt. Sowohl Zonenelektrophorese als auch mizellare elektrokinetische Chromatographie mit Trägerelektrolyten bestehend aus Boratpuffern (*pH* 9 bis 9.4) mit oder ohne Natriumdodecylsulfat wurden für die Trennung eingesetzt. Eine Optimierung der Trennselektivität war durch die Zugabe von bis zu 20% 2-Propanol oder Methanol zum Trägerelektrolyten möglich. Quantifizierung mittels interner Standards ergab relative Standardabweichungen zwischen 0.2 und 0.8%. Nebenprodukte konnten in technischen Proben bis zu Gehalten von 0.1% analysiert werden.

Introduction

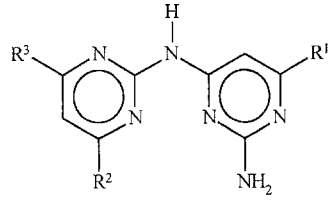
Substituted pyrimidines are important building blocks for the production of various agrochemicals. Among others, compounds **1c** and **1l** (Scheme 1) are used for the manufacturing of important herbicides and fungicides [1]. Pyrimidine derivative **1c** is synthesized from diethylmalonate *via* **1a**, **1l** from diethylmalonate *via* **1e** and **1g**. Quality control in the manufacturing process requires appropriate analytical methods for the determination of the purity of each product as well as the content of various by-products. Currently employed analytical techniques include titrimetry, gas chromatography (GC), and high-performance liquid chromatography

(HPLC) [2, 3]. All of them suffer from various drawbacks. The selectivity of titrimetry is poor; GC can be used for the analysis of **1c** and **1g**, but even in these two cases some of the possibly present by-products show low volatility and decompose during the GC run. HPLC suffers from long separation times; as the polarities of the components of a sample differ considerably, the analytes can be separated only by gradient elution which leads (including the equilibration of the column) to experiment duration of up to eighty minutes.



1a-o

	R^1	R^2	R^3	R^4
1a	H	OH	H	OH
1b	H	Cl	H	OH
1c	H	Cl	H	Cl
1d	H	Cl	Cl	Cl
1e	NH_2	OH	H	OH
1f	NH_2	Cl	H	OH
1g	NH_2	Cl	H	Cl
1h	NH_2	Cl	Cl	Cl
1i	NH_2	Cl	H	$N(C_2H_5)_2$
1j	NH_2	OH	H	OCH_3
1k	NH_2	Cl	H	OCH_3
1l	NH_2	OCH_3	H	OCH_3
1m	NH_2	OCH_3	Cl	OCH_3
1n	NH_2	OCH_3	H	$N(C_2H_5)_2$
1o	OCH_3	OCH_3	H	OCH_3



2a-b

	R^1	R^2	R^3
2a	Cl	Cl	Cl
2b	OCH_3	OCH_3	OCH_3

Scheme 1

Capillary electrophoresis (CE) can be an attractive alternative to chromatographic techniques; so far, however, it seems that it has not yet been applied to the analysis of pyrimidine compounds. Nowadays, CE is well accepted by the analytical chemist; on the other hand, its introduction to industrial product control is still at the beginning. The aim of the work presented in this paper was the investigation of the capillary electrophoretic behaviour of substituted pyrimidines and the applicability of electrophoresis to the industrial analytical laboratory.

Results and Discussion

Separation of compounds in technical samples of **1l**, **1g**, *and* **1c**

Compounds **1l**, **1g**, **1c** and similar substances can be converted into cationic forms after protonation of the ring nitrogen or an amino group. Unfortunately,

pK_b values were available only for a few substituted aminopyrimidines [4], making it difficult to predict the *pH* of a carrier electrolyte that would allow a separation of all compounds under investigation in the mode of capillary zone electrophoresis. For this reason, we decided to carry out the separation of the analytes as uncharged species by micellar electrokinetic chromatography (MEKC).

Recently, MEKC has successfully been employed for the analysis of barbiturates using phosphate and borate buffers containing sodium dodecylsulfate (*SDS*) as carrier electrolytes [5]. Because of some similarities between barbiturates and some of the pyrimidines under investigation, phosphate and borate buffers in a *pH* range of 7.5 to 11.5 containing *SDS* in concentrations from 35 m*M* to 150 m*M* were investigated in a series of priliminary experiments for the analysis of technical samples of **1l**, **1g**, and **1c**. Detection was done at the cathodic side. Unfortunately, a separation of all compounds of interest in these samples could not be achieved. A possible approach to the optimization of separation selectivity is the use of organic solvents in the carrier electrolyte [6–12]. There are several effects that can be achieved in this way [11, 12]: the electroosmotic mobility is reduced so that the time window available for the separation (*i.e.*, the time between an analyte migrating with the electroosmotic flow and an analyte migrating with the negatively charged micelle) is increased; the distribution of analytes between the micelles and the "aqueous" phase is altered; and, finally, organic solvents can have a significant impact on the structure of the micelles. A prediction of the overall influence of organic solvents on migration times and separation selectivity turned out to be difficult; the effect of the decreased electroosmotic flow, for example, can be counteracted by the decreased interaction of the analytes with the micelles. Experimental work was therefore carried out with different organic solvents including acetonitrile, methanol, ethanol, 2-propanol, and 2-butanol.

Practically all organic solvents used in the carrier electrolytes for the separation of main products and by-products in technical samples of **1l**, **1g**, and **1c** yielded significant changes in separation selectivity. The most promising solvents were 2-propanol for samples of **1l** and methanol for those of **1g** as well as **1c**. The dependencies of migration times upon the percentage of organic modifier are shown in Figs. 1 to 3.

It must be kept in mind that some of the by-products present in the technical products can be anionic at the *pH* of the borate carrier electrolyte (for example compound **1j** present in samples of **1l**). A decrease of the *pH* will decrease the ionization and shorten the migration times of such species. Selection of an appropriate *pH* value of the carrier electrolyte could therefore be successfully employed for fine-tuning of the separation.

Optimum separation selectivity was achieved when the basic carrier electrolyte (*i.e.*, 25 m*M* sodium tetraborate and 50 m*M* *SDS*) was modified with 20% 2-propanol and adjusted to *pH* 9.4 for samples of **1l**, with 10% methanol and adjusted to *pH* 9 for samples of **1g**, and with 10% methanol without further *pH* adjustment for samples of **1c**. Typical electropherograms of samples of the pyrimidines **1l**, **1g**, and **1c** containing the whole range of relevant by-products are shown in Figs. 4 to 6.

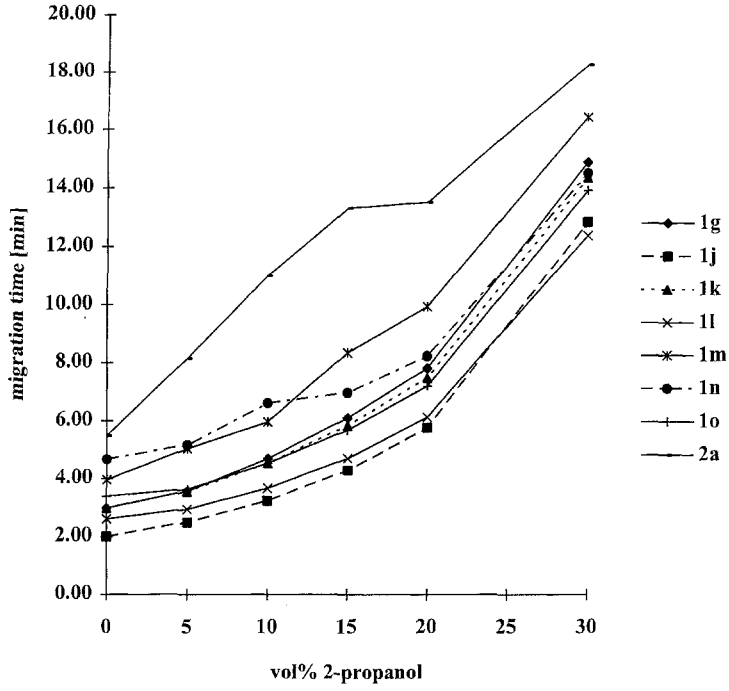


Fig. 1. Dependence of the migration times of the main product **1l** and by-products upon the percentage of 2-propanol in the carrier electrolyte consisting of 25 m*M* sodium tetraborate / 50 m*M* *SDS*

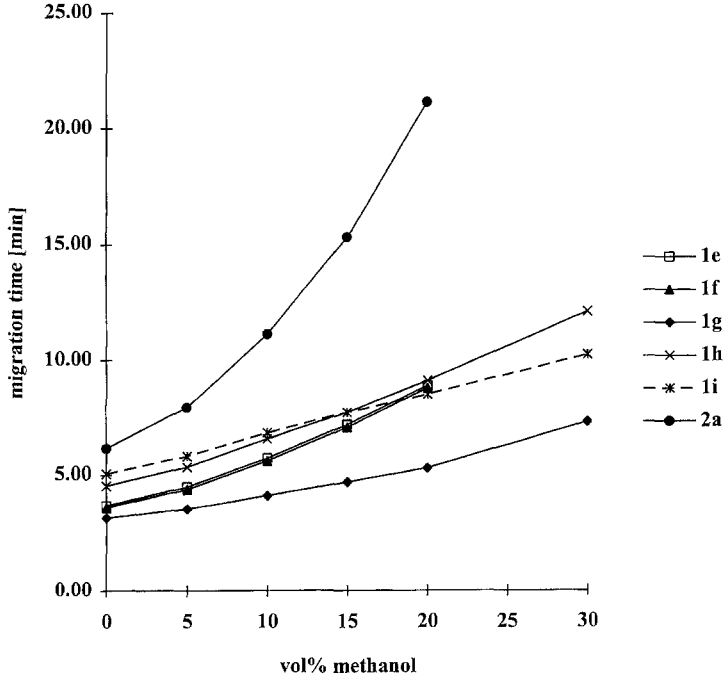


Fig. 2. Dependence of the migration times of the main product **1g** and by-products upon the percentage of methanol in the carrier electrolyte consisting of 25 m*M* sodium tetraborate / 50 m*M* *SDS*

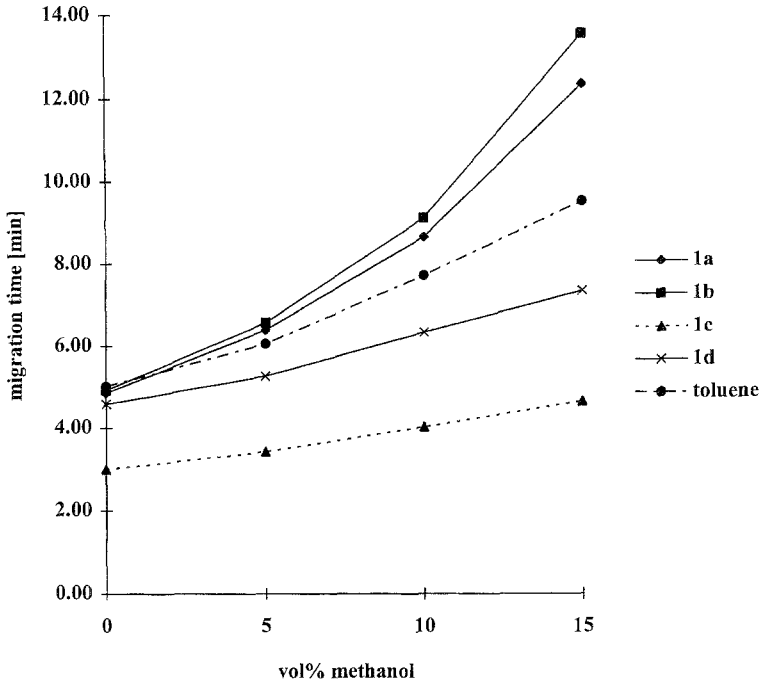


Fig. 3. Dependence of the migration times of the main product **1c** and by-products upon the percentage of methanol in the carrier electrolyte consisting of 25 m*M* sodium tetraborate / 50 m*M* *SDS*

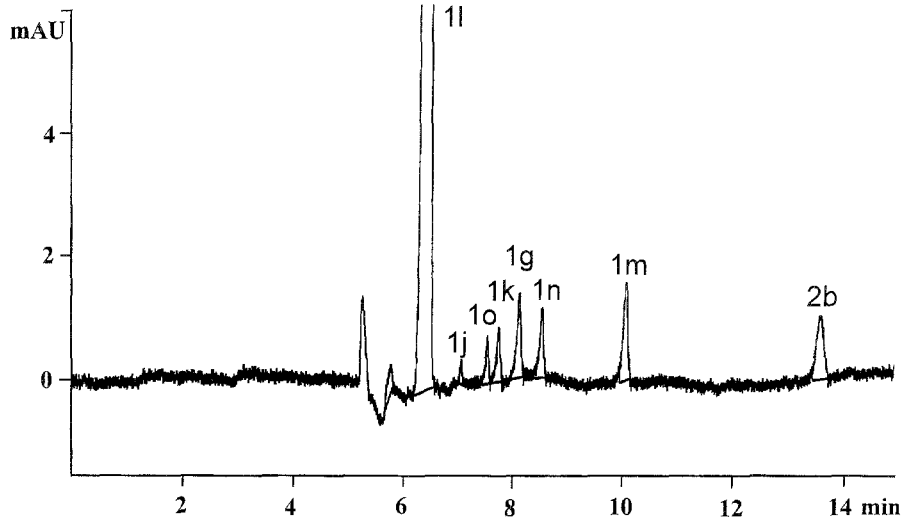


Fig. 4. Electropherogram of a sample of **1l** containing about 0.1% (m/m) of each by-product; buffer: 25 m*M* sodium tetraborate / 50 m*M* *SDS* containing 20% (v/v) 2-propanol, *pH* 9.4; UV detection at 240 nm

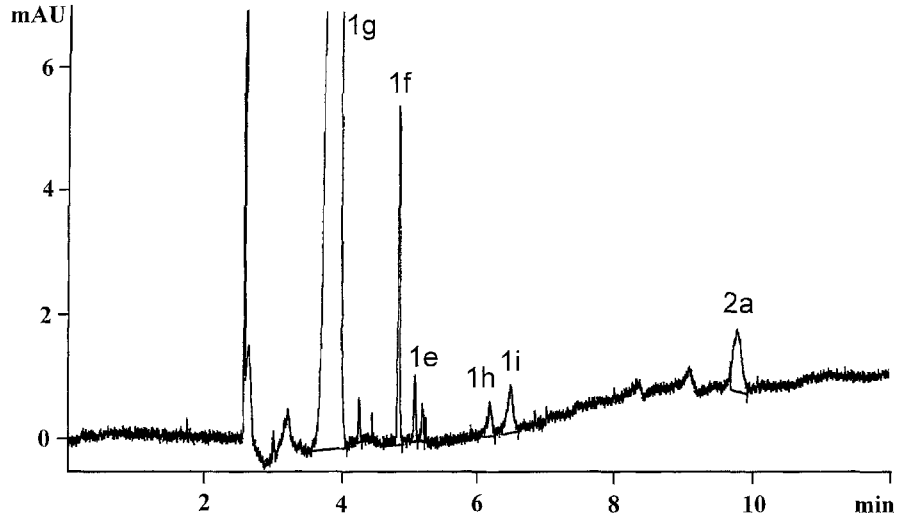


Fig. 5. Electropherogram of a sample of **1g** containing about 0.8% (m/m) of **1f**, 0.2% (m/m) of **2a**, and 0.1% (m/m) of the other by-products; buffer: 25 m*M* sodium tetraborate / 50 m*M SDS* containing 10% (v/v) methanol, *pH* 9.0; UV detection at 240 nm

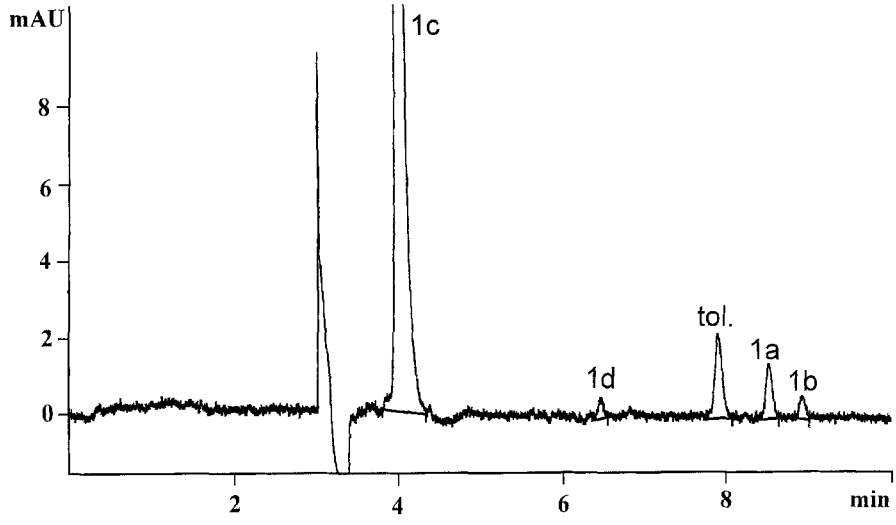


Fig. 6. Electropherogram of a sample of **1c** containing about 0.3% (m/m) of toluene and 0.1% (m/m) of the other by-products; buffer: 25 m*M* sodium tetraborate / 50 m*M SDS* containing 10% (v/v) methanol, *pH* 9.3; UV detection at 200 nm

Separation of compounds in technical samples of **1a** *and* **1e**

The by-products in technical samples of **1a** and **1e** were mainly nonionic compounds like esters or amides of short-chain carboxylic acids. All attempts to

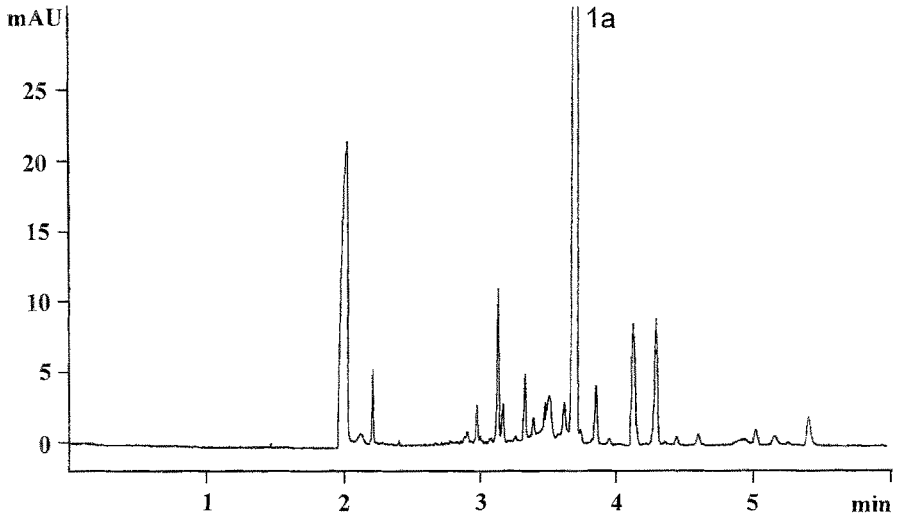


Fig. 7. Electropherogram of a sample of **1a** after storage at elevated temperature; carrier electrolyte: 25 m*M* sodium tetraborate; UV detection at 210 nm

separate these by-products by MEKC failed; obviously, their polarity is too high for an interaction with micelles. For this reason, method development for technical samples of **1a** and **1e** was restricted to the determination of the main product. The pK_a values of the hydroxy groups of **1a** and **1e** amount to about 4 and 9.5; therefore, capillary zone electrophoresis with borate buffers as carrier electrolytes was chosen which allowed an easy separation of the main product from non-ionic by-products. At the same time, it was possible to separate the main product **1a** from a range of unknown by-products generated during stability tests in aqueous solution at elevated temperature (Fig. 7). It is assumed that compound **1a** decomposes in water at elevated temperatures to form dimethylmalonate and formic acid as the main products.

Method validation

For validation, the following parameters were investigated: the repeatability of the analysis, the linearity of the signals for the main products and the by-products, and the recoveries of the by-products.

When evaluating the repeatability of peak areas, one should keep in mind that the repeatibility of the injection was not better than 2% relative standard deviation. This appeared adequate for the analysis of by-products in technical samples at levels of 0.2% and less. On the other hand, some improvement seemed necessary for the analysis of the main component in a technical sample; this led to the use of internal standards (diethylphthalate for **1l**, 3,5-dinitrobenzoic acid for **1g**, chinaldinic acid for **1c**, 2,4,6-trihydroxypyrimidine for **1a** and **1e**). In addition, peak areas were divided by their migration times to correct small variations in areas due to variations in migration times. In this way, relative standard deviations

Table 1. Detection wavelengths and detection limits of the by-products in samples of **1l**, **1g**, and **1c**

	Detection wavelength (nm)	Detection limit (mg/l)		Detection wavelength (nm)	Detection limit (mg/l)
1a	200	0.8	**1j**	230	2.7
1b	200	0.8	**1k**	230	1.4
1d	200	1.1	**1m**	240	1.4
1e	215	0.9	**1n**	215	1.1
1f	215	1.1	**1o**	240	1.9
1g	230	1.5	**2a**	240	2.3
1h	240	2.1	**2b**	230	1.6
1i	215	1.8	Toluene	200	3.5

for the quantification of the main products between 0.2 and 0.8% ($n = 10$) could be achieved for the quantification of main products.

Linearity of the signals for the main products in technical samples was checked from 40 to 200% of the expected concentration. Using the ASTM criterion [13], linearity was fulfilled in all cases.

In the same way, linearity was checked for all by-products possibly present in technical samples from the detection limit up to concentrations corresponding to a content of 0.3% (higher concentrations were irrelevant with respect to product specifications). All calibration graphs were found to show a linear behaviour.

Detection limits (signal-to-noise ratio of 3) and detection wavelengths for all by-products investigated in the present work are summarized in Table 1.

Recoveries of the by-products were determined by repeated addition of known amounts of each substance followed by external quantification. For the by-products in **1l** and **1g**, recoveries between 90% and 105% were found. For the by-products in **1c**, recoveries were of the same order except for toluene yielding a recovery of only 81% and for compound **1b** yielding 113%. The reason for the poor recovery of toluene was assumed to be the high volatility of this compound resulting in pour precision of the quantification. A possible explanation for the extraordinary high recovery of **1b** could be a different spectral absorbance of this compound in different runs due to small changes in the *pH* value of the buffer system between the runs (the pK_a value of this substance is in the same order of magnitude as the *pH* of the buffer).

Another parameter checked during method validation was the stability of the solutions which were prepared corresponding to the procedure described below. The stability of the main products as well as the by-products was checked after 3, 7, and 10 days by external calibration using freshly prepared standard solutions each time. The maximum decrease of the content did not exceed 5% except for toluene which decreased by 60% due to evaporation during the storage over 10 days.

The results obtained in this work indicate that capillary electrophoresis is a reliable technique for quality control of technical samples of various pyrimidine compounds. High efficiencies and short analysis times are some of the advantages of this method compared to the well-established technique of high-performance

liquid chromatography. Therefore, capillary electrophoresis appears to be an attractive alternative for routine applications.

Experimental

Instrumentation

The CE instrument employed was a HP 3D Electrophoresis System (Hewlett Packard, Palo Alto, CA, USA) equipped with a diode array detector and interfaced to a 3D CE Chemstation (Hewlett Packard). Separations were carried out using fused silica capillaries with a total length of 48.5 cm, an effective length of 40 cm, and an inner diameter of 50 μm. At the point of detection, the inner diameter of the capillary was increased to 150 μm (bubble cell). Injection was performed by applying a pressure of 50 mbar to the sample vial for 5 seconds.

Carrier electrolytes

The carrier electrolyte used for the analysis of samples of dihydroxypyrimidines **1a** and **1e** was a 25 m*M* solution of sodium tetraborate. Samples of **1l**, **1g**, and **1c** were analyzed in a carrier electrolyte consisting of 25 m*M* sodium tetraborate and 50 m*M* sodium dodecylsulfate (*SDS*). To achieve appropriate separations of the by-products, addition of 20% (v/v) 2-propanol and the adjustment of the *pH* to 9.4 were necessary for the analysis of **1l**. For the analysis of **1g** and **1c**, 10% (v/v) methanol as organic modifier showed the best results. In the case of the analysis of **1g**, the buffer had to be adjusted to *pH* 9.0. The buffer solution for the analysis of **1c** required no further adjustment of *pH*. All carrier electrolytes were prepared from analytical reagent grade chemicals using water purified with a Millipore (Bedford, MA, U.S.A.) Milli-Q water treatment system.

Preparation of samples and standard solutions for **1a** *and* **1e**

Internal standard solutions for the analysis of both **1a** and **1e** were prepared by dissolving 200 mg 2,4,6-trihydroxypyrimidin in 100 ml of deionized water.

For the analysis of samples of **1e**, 20 mg of a standard of **1e** were dissolved in 5 ml of 0.1 *N* NaOH, mixed with 10 ml internal standard solution, and diluted to 100 ml with water. The technical samples of **1e** were treated in the same way.

Standard solutions of **1a** were prepared by dissolving 20 mg of a standard of **1a** in about 70 ml water, mixing with 10 ml internal standard solution, and diluting to 100 ml with water.

Preparation of samples and standard solutions for **1l**

For the quantification of the by-products in **1l**, a standard stock solutions was prepared by dissolving 10 mg of **1g**, **1j**, **1k**, **1m**, **1n**, **1o**, and **2b** each in 30 ml acetonitrile and adding water to 100 ml. 10 ml of this stock solution were mixed with 27 ml acetonitrile and 50 ml of a 50 m*M* *SDS* solution and diluted to 100 ml with water.

The sample solutions for the analysis of the by-products were prepared by dissolving 300 mg of the technical sample of **1l** in 30 ml acetonitrile, adding 50 ml of a 50 m*M* solution of *SDS* and diluting with water to 100 ml.

For the quantification of **1l**, standard solutions were prepared from 15 mg of **1l** dissolved in 5 ml acetonitrile; after the addition of 50 ml of 50 m*M* *SDS* solution, the mixture was made up to 100 ml with water. Sample solutions of **1l** were prepared by mixing 5 ml of the solution which was used for the analysis of the by-products with 5 ml of internal standard solution (300 mg diethylphthalate in

30 ml acetonitrile, water added to 100 ml). After the addition of 50 ml of a 50 m*M SDS* solution, the mixture was diluted to 100 ml with water.

Preparation of samples and standard solutions for **1g**

Sample and standard solutions were prepared as described for the analysis of **1l** using compounds **1f**, **1h**, **1i**, and **2a** as standards for the quantification of the by-products. Standard solutions of **1e** had to be prepared separately by dissolving 10 mg of **1e** in 5 ml 0.1 *N* NaOH and adding water to 100 ml.

For the quantification of **1g**, 3,5-dinitrobenzoic acid was used as internal standard following the procedure described for the analysis of **1l**.

Preparation of samples and standard solutions for **1c**

Compounds **1a**, **1b**, **1d**, and toluene were used as standards for the analysis of by-products in **1c**. For a standard stock solution, 10 mg of each substance were dissolved in 30 ml acetonitrile. In order to lower the *pH* of the solution, which was necessary to increase its stability, 0.6 ml of concentrated phosphoric acid were added and the solution was diluted to 100 ml with water. For the standard solution, 10 ml of this stock solution were mixed with 27 ml acetonitrile and 0.6 ml phosphoric acid. After the addition of 50 ml of a 50 m*M SDS* solution, the mixture was diluted to 100 ml with water.

The sample solution for the quantification of **1c** was prepared in the same way as described above for the analysis of **1l** except for the addition of 0.6 ml of concentrated phosphoric acid. Chinaldinic acid was used as internal standard.

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