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Capillary Supercritical Fluid Chromatography/Mass Spectrometry of Phenolic Mannich Bases with Dimethyl Ether Modified Ethane as the Mobile Phase

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The analysis of phenolic Mannich bases—which are used as hardeners and accelerators for epoxy resins—by capillary supercritical fluid chromatography (SFC) with dimethyl ether modified ethane as the mobile phase is described. The elution properties of several different mobile phases with respect to amines are shown. SFC with UV detection is coupled via a custom-built interface to a mass spectrometer with atmospheric pressure chemical ionization. Two technically important Mannich bases prepared by different production processes are characterized and compared with respect to their byproducts. The role of dimethyl ether during the ionization process and the fragmentation of phenolic Mannich bases is discussed.

Phenolic Mannich bases have been used for more than 40 years as hardeners and accelerators for epoxy resins.[1] Their outstanding properties make them ideal cold curing agents for civil engineering and coating applications.[2] In recent years, stricter environmental regulations with respect to volatile organic compounds for coatings have led to a renewed interest in this important substance class.[3,4] Despite the fact that several thousand tons of phenolic Mannich bases are fabricated every year, analytical techniques for the characterization of these widely applied products are scarce. The literature describes the use of gas chromatography (GC) only for the determination of residual phenols, formaldehyde, and formulation components[5,6] in Mannich bases, whereas thin-layer chromatography is used to follow the Mannich reaction[7] and to determine residual free amine.[8] No chromatographic method for the separation of individual components of phenolic Mannich bases has been described in the literature. Low volatility due to high polarity and the higher molecular weight of byproducts, as well as thermal instability, prevents separation by GC except in some specific cases. In addition, derivatization of amine functionalities is necessary to make these substances amenable to high-performance liquid chromatography (HPLC). The instability of phenolic Mannich bases, or at least of byproducts that may be present in such Mannich bases, makes it difficult to choose a derivatization method that does not affect the composition of the original products and thus impedes the use of HPLC for their characterization. Capillary supercritical fluid chromatography (SFC) is the only chromatographic technique that allows excellent separation in inert media and at relatively mild temperatures, both prerequisites for the successful separation and characterization of phenolic Mannich bases. In addition, capillary SFC can be readily coupled to mass spectrometry (MS), yielding reliable structural information of unknowns. Unfortunately, capillary SFC has only a modest track record for the analysis of polar amines or amine-based compounds. Carbon dioxide (CO_2)—as the most common mobile phase in capillary SFC—elutes only amines with relatively low basicity.[9] The literature is somewhat contradictory regarding this effect, but the formation of insoluble carbamates seems to be the main reason for this behavior.[10−12] Sulfur hexafluoride (SF_6), in contrast, only

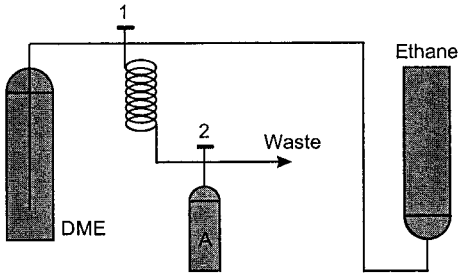

Figure 1. Scheme for the preparation of the mobile phase (for details, see text).

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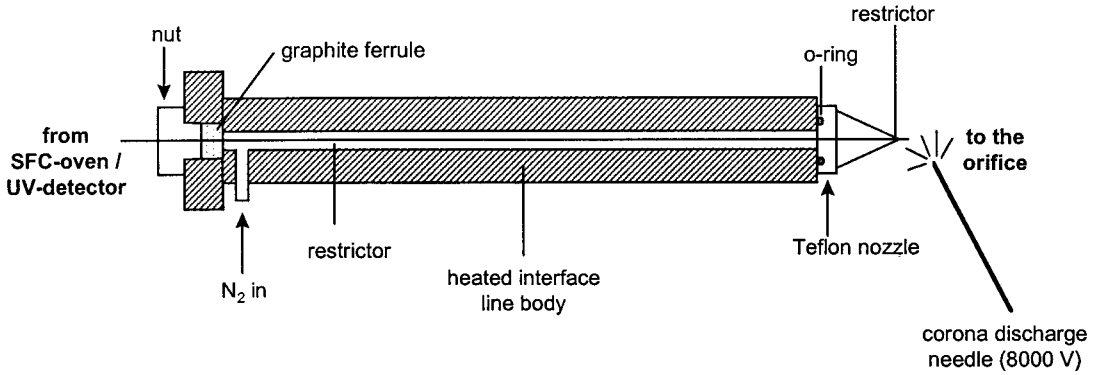

Figure 2. SFC/MS interface. The eluent is passed through the UV detector into the restrictor. The restrictor is held in place by the graphite ferrule in the back of the interface. Nitrogen is fed into the resistively heated interface, flowing out coaxially to the restrictor through the Teflon nozzle, which is placed just above the corona discharge needle.

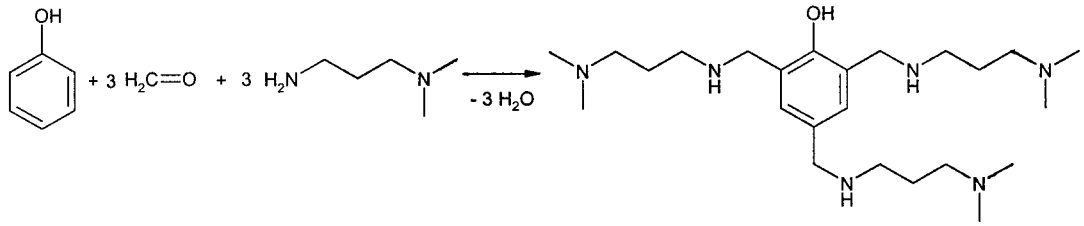

Figure 3. Preparation of MB-1 by the classical Mannich reaction.

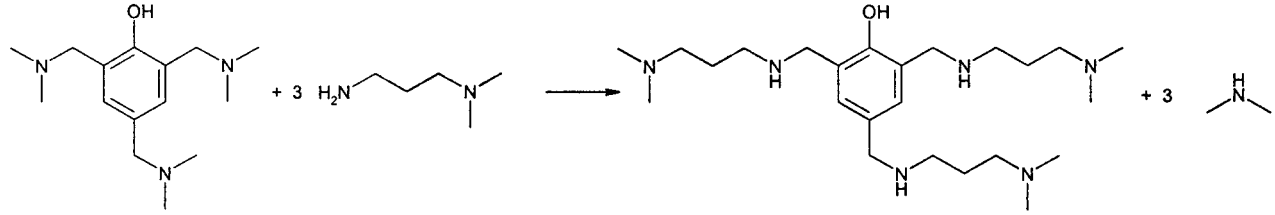

Figure 4. Preparation of MB-2 by transamination.

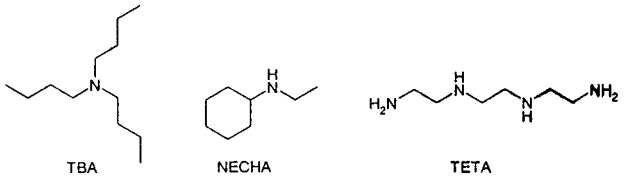

Figure 5. Constituents of the amine test mixture.

elutes monofunctional amines,[13] and laughing gas (N_2O), despite its higher polarity, was also only described for the elution of monofunctional amines by capillary SFC.[14,15]

As no further mobile phases for the separation of amines by capillary SFC are reported in the literature, this paper first describes the development of a capillary SFC method for the elution of highly polar multifunctional amines, which is then applied for the characterization of phenolic-based Mannich bases by SFC with UV detection and SFC-UV coupled to MS. Identification of main products and byproducts is readily achieved using these methods, allowing even the identification of the manufacturing process of phenolic Mannich bases.

EXPERIMENTAL SECTION

Supercritical Fluid Chromatography. A Fisons Instruments SFC system (Fisons Instruments SpA, Rodano, Italy) consisting of an SFC 300 double-syringe pump and an SFC 3000 series oven equipped with an AS 500 autosampler and an OL 407 injector valve (Valco Instruments. Co. Inc., Houston, TX) was used. The pump was kept at 5 °C, and the injector, at 28 °C. The 1 μL sample loop was kept for 300 ms on the inject position. An approximate split ratio of 1:1 was achieved using a splitter (Fisons Instruments; series no. 34708440) equipped with a 1 m split column (Composite Metal Services Ltd., Worcester, U.K.; i.d. 75 μm, series no. TSP075150). Separation was performed on a DB-1 fused-silica capillary column (J&W Scientific, Folsom, CA) with a length of 20 m, an i.d. of 0.1 mm, and a 0.2 μm film at 130 °C. For detection, a UV 1000 detector (Thermo Separation Products, San Jose, CA), equipped with an SFC-UV cell (Linear Instruments Corp., Reno, NV; series no. 2520-4206) was used. Restriction was performed by a 1 mL/min integral restrictor (J&W Scientific; series no. 009-0202SP, length 1 m). Density programming over the whole range of the apparatus was used for all mobile phases. The density program for the dimethyl ether (DME)/ethane mixture was as follows: 15 min at 0.14 g/mL, with a gradient of 0.02 (g/mL)/min to 0.42 g/mL, 5 min at 0.42 g/mL.

Preparation of the Mobile Phase. The mobile phase was prepared by mixing liquid dimethyl ether (SL Gas, Lenzburg, Switzerland; > 99.8%) and liquid ethane (Carbagas, Bern, Switzerland; >99.95%) in a 1 L gas bomb (see Figure 1). Therefore the three-port valves (Swagelok Co., Solon, OH; series no. SS-

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(14) Baastoe, M. B.; Lundanes, E. *J. Chromatogr.* **1991**, *558*, 458−463.
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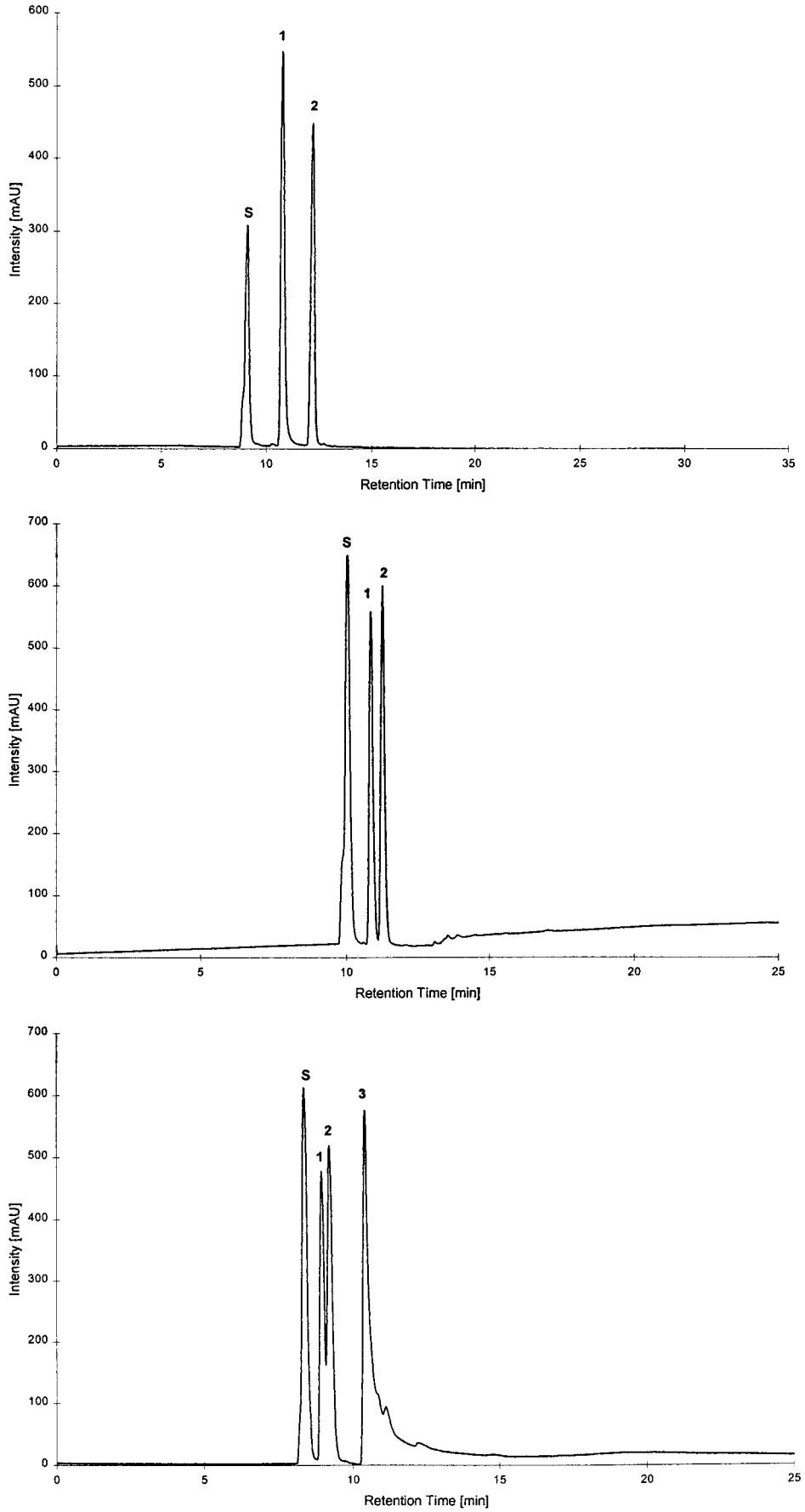

Figure 6. SFC with UV detection (200 nm) of the test mixture with CO_2 (top), ethane (middle), and 24% (mol/mol) of dimethyl ether in ethane (bottom).

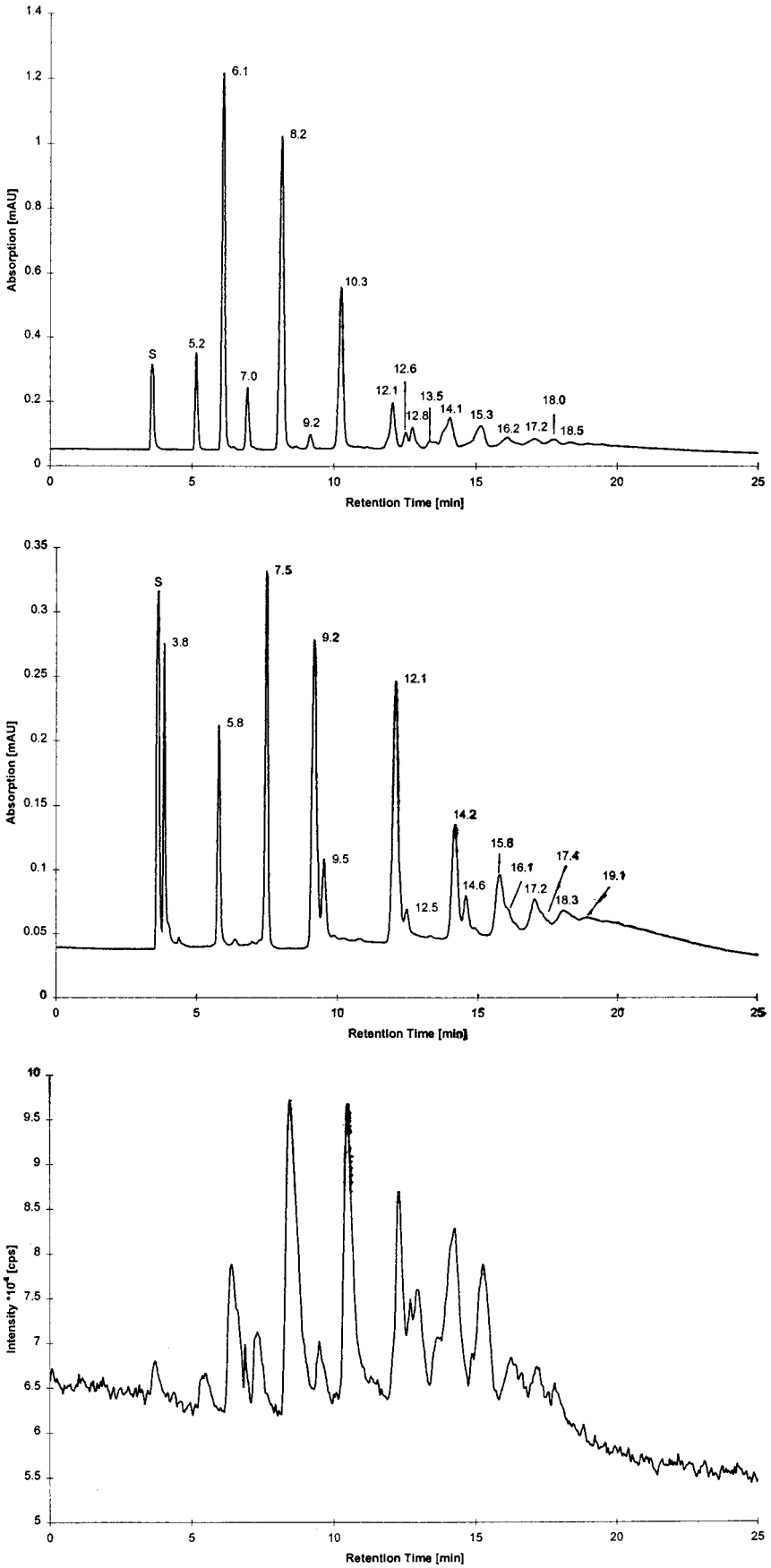

Figure 7. SFC with UV detection (200 nm) of MB-2 (top) and MB-1 (middle). Bottom: corresponding SFC with MS detection of MB-1.

41XTS2) 1 and 2 were opened to flush the stainless steel coil (length 16 m, i.d. 2.35 mm, volume 70 mL) with liquid DME.

After valve 1 was closed, valve 2 was opened to fill the gas bomb with liquid DME. For a better transfer of the liquid DME

into the ice-cooled gas bomb, the coil was heated to about 60 °C using a heat gun. The quantity of transferred DME was controlled by weight. The filling was repeated until the necessary amount of DME was transferred into the bomb. The corresponding amount of ethane was transferred using the same procedure.

Research grade sulfur hexafluoride, laughing gas, and carbon dioxide were obtained from Scott Specialty Gases (Plumsteadville, PA). Xenon (>99.99%) was obtained from SL Gas, Lenzburg, Switzerland. Xenon was transferred from the gas reservoir into the pump as described in the literature.[16]

SFC/MS. A Perkin-Elmer Sciex API *III*+ triple-quadrupole mass spectrometer with an atmospheric pressure chemical ionization device (Perkin-Elmer Sciex, Toronto, Canada) and a custom-built interface (see Figure 2) was used for SFC/MS experiments.

Nebulization gas (nitrogen >99.999%) pressure was held at 140 kPa (20 psi, resulting in a flow of 0.6 L/min). If not mentioned otherwise, MS conditions were as follows. For positive chemical ionization, the corona discharge needle supplied a discharge current of ~3 μA at 8000 V. The interface plate was held at 650 V, and the orifice, at 75 V. The curtain gas flow (nitrogen >99.999%) was maintained at 0.8 L/min. The first quadrupole of the mass spectrometer was scanned with a step rate of 0.4 between m/z 140 and 1000, a dwell time of 0.97 ms, and a pause time of 0.02 ms, resulting in 0.47 scan/s.

Reagents and Samples. Tributylamine, *N*-ethylcyclohexylamine, and triethylenetetramine (technical grade) were received from Fluka, Buchs, Switzerland. If not stated otherwise, 1% solutions in a 1:1 mixture of methanol and dichloromethane (both from Fluka) were prepared and injected as such. The Mannich bases characterized were prepared as follows. Mannich base 1 (MB-1) was prepared by the Mannich reaction of phenol, *N*,*N*-dimethyl-1,3-propanediamine, and formaldehyde (36% solution in water) (all from Fluka) (see Figure 3). Mannich base 2 (MB-2) was prepared by transamination of 2,4,6-tris(dimethylaminomethyl)phenol (Ciba Specialty Chemicals, Basel, Switzerland) with *N*,*N*-dimethyl-1,3-propanediamine (see Figure 4). Solutions of MB-1 and MB-2, 1% (w/w), were prepared in methanol puriss. (Fluka) and injected as such.

RESULTS AND DISCUSSION

The initial goal was to develop a capillary SFC method for the separation of polar phenolic Mannich bases. An amine mixture consisting of tributylamine [102-82-9] as tertiary amine, *N*-ethylcyclohexylamine [5459-93-8] as secondary amine and triethylenetetramine (TETA [112-24-3]) as highly polar polyamine was used to evaluate the elution power of different mobile phases with respect to amines (see Figure 5).

Due to their different functionalities, these amines allow a good assessment of each mobile phase for the elution of amines. Additionally, TETA shows amine functionalities similar to those of a multifunctional Mannich base, making it a good indicator for the applicability of the mobile phase for their separation. As can be seen in Figure 6 and in agreement with the literature, CO_2 only elutes the secondary and tertiary amines of the test mixure; TETA as a multifunctional amine is not eluted. Additional investigations have shown that monofunctional primary amines, such as decylamine and dodecylamine, can be eluted with CO_2, supporting the theory that only multifunctional primary amines are not eluted by CO_2.[14,15] Despite their inability to form carbamates, N_2O and xenon yield similar results, eluting the tertiary and secondary amines of the test mixture, as well as decyl- and dodecylamine, but not eluting TETA. The failure to elute TETA and poor chromatographic resolution eliminate SF_6 as a mobile phase for phenolic Mannich bases. And ethane, as an inert alkane with suitable critical values, does elute the tertiary and secondary amines but again fails to elute TETA.

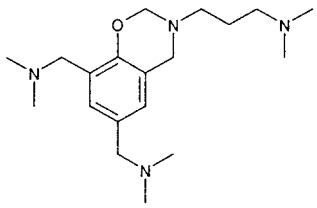

Figure 8. Oxazine derivative of the compound eluting at 8.2 min (see Table 1).

Trials to increase the elution power of CO_2 through the addition of polar modifiers such as methanol or 2-propanol did not yield any useful results, as they quickly destroyed the column, resulting in clogging of the restrictor and irreproducible results. As a consequence, inert but less polar modifiers such as tetrahydrofuran (THF) and dimethyl ether (DME) were tested for the separation of the amine mixture. The high critical temperature of THF (267.3 °C) and probably related but not further investigated problems with UV detection resulted in the use of DME as the modifier. For reasons of miscibility, and to avoid the formation of carbamates, a mixture of DME with ethane was preferred to a mixture of DME with CO_2. Several mixtures were prepared with different mixing ratios, and although 11% (mol/mol) DME in ethane elutes TETA, a mixture with 24% DME performed best with respect to elution power and separation for the compounds described below. As can be seen in Figure 6, it gives reasonable separation of the three test compounds as well.

Although the use of mobile phases with higher amounts of DME may be necessary for the elution of amines with higher polarity or higher molecular weight compounds and a lower concentration of DME may yield better separations for less polar products, the mixture with 24% gave excellent results in most cases and was used throughout this work. A 100% dimethylsiloxane stationary phase was preferred to higher polar stationary phases such as DB-17 (50% diphenylsiloxane) due to its higher resistance against the supercritical mobile phase.

The application of this SFC method to the separation of the constituents of technical phenolic Mannich bases reveals a multitude of different products and byproducts (see Figure 7) and, even more importantly, huge differences between so-called identical Mannich bases (Mannich bases consisting of the same phenol and the same amine). Although differences in application-related properties, such as viscosity and reactivity, can be detected between these Mannich bases, investigations regarding differences in their structural composition have not previously been carried out.

In this case, SFC-UV coupled to MS is the method of choice for the identification of unknowns. An SFC/MS interface for atmospheric pressure chemical ionization (APCI) similar to the

(16) Kirschner, C. H.; Taylor, L. T. *J. High Resolut. Chromatogr.* **1994**, *17*, 61−67.

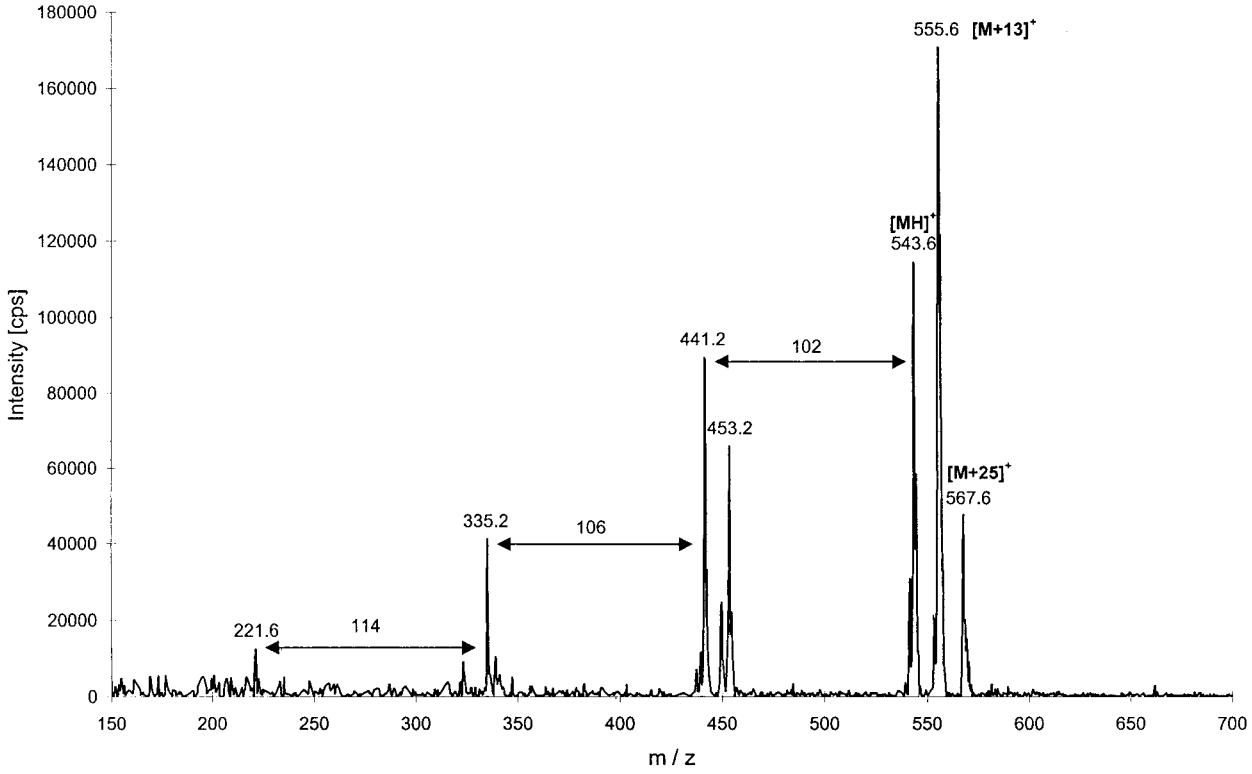

Figure 9. Mass spectrum of the compound eluting at 14.2 min. In addition to the $[MH]^+$ ion, $[M + 13]^+$ and $[M + 25]^+$ ions are formed. The fragmentation confirms the structure.

one described by Tyrefors et al.[17] was used (see Figure 2). Positive APCI allows the detection of virtually all eluting components without any addition of water or other protonating agent. The use of water-saturated nitrogen as the nebulizing gas did not result in a higher ion yield or a better signal-to-noise ratio. Astonishingly, the chromatographic resolution seems not to be influenced by the temperature of the transfer line between the SFC oven and the MS, a fact also observed by Morgan et al.[18] The chromatographic resolution at a transfer line temperature of 100 °C is not better than that at ambient temperature; the same effect was observed for a heated and an unheated UV flow cell, as long as the tip of the restrictor was heated to compensate for the Joule–Thompson effect. The coaxial introduction of nitrogen through the heated interface is sufficient to compensate for the cooling due to expansion of the mobile phase.

The low chemical background due to high-purity mobile phases in SFC/MS allows straightforward detection of very low molecular weight compounds. Figure 7 shows the corresponding SFC with MS detection of one of the Mannich bases. Good signal-to-noise ratios and similar relative intensities compared to those of UV detection allow an easy correlation between UV and MS detection.

The fact that DME is a useful if unusual positive ion reagent for chemical ionization[19] is a further advantage of this mobile phase and is probably the main reason for the good ion yield without the addition of any protonating agent. As described in the literature,[20] mainly protonated and $[M + 13]^+$ ions were observed. The $[M + 13]^+$ ion forms only in cases where a secondary aminomethyl function is present ortho to the phenolic hydroxyl group. In cases where two such secondary *o*-aminomethyl groups are present, even the $[M + 25]^+$ ion is formed (see Figure 9). For this reason, it can be assumed that the $[M + 13]^+$ and $[M + 25]^+$ ions are not real adduct ions but protonated oxazine derivatives (see Figure 8), formed by alkylation of the Mannich base and subsequent cyclization.

Typical fragmentation includes the elimination of the amine, and sometimes the elimination of the whole aminomethyl moiety, thus providing an excellent way to distinguish between Mannich bases with different substituents. The spectrum in Figure 9 clearly shows the elimination of the amine ($\Delta = 102$), the subsequent loss of the methylphenol moiety ($\Delta = 106$), and the loss of the resulting methyl derivative of the amine ($\Delta = 114$) of the former bridge. The fragment at m/z 453 confirms the formation of the oxazine. Variation of the orifice voltage effects control of the fragmentation; a low orifice voltage (e.g. 35 V) yields mainly $[MH]^+$ and $[M + 13]^+$ ions, whereas a high orifice voltage (e.g. 150 V) gives mainly fragment ions. An orifice voltage of 75 V seems to be the best compromise for the formation of both protonated and fragment ions.

An important application of this method is the characterization of technically important Mannich bases with respect to their byproducts. This allows the optimization of production processes, as well as the differentiation between similar Mannich bases prepared by different synthetic pathways. Table 1 lists the structural proposals for two similar Mannich bases (for chromato-

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(18) Morgan, D. G.; Harbol, K. L.; Kitrinos, N. P., Jr. *J. Chromatogr.* **1998**, *800*, 39–49.

(19) Burrows, E. P. *Mass Spectrom. Rev.* **1995**, *14*, 107–115.

(20) Burrows, E. P. *J. Mass Spectrom.* **1998**, *33*, 221–228.

Table 1. Structural Proposals for the Peaks Labeled in Figure 7[a]

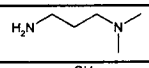
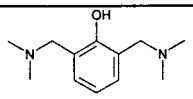
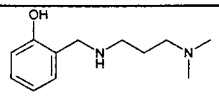
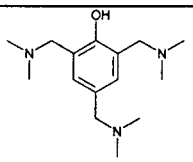
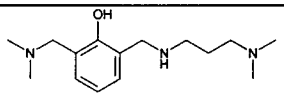
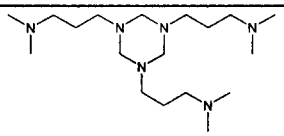
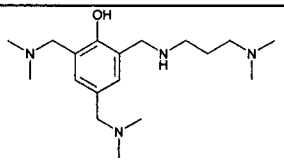
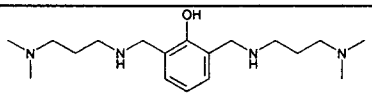
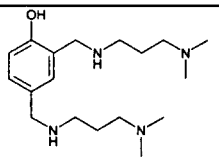
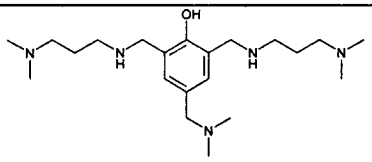
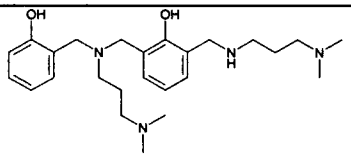
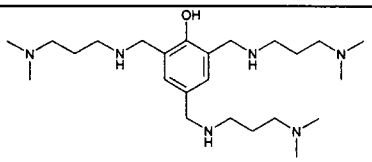
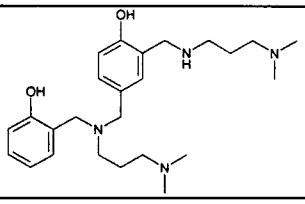
Retention time [min]	Structural proposal	Molecular weight [g/mol]	Detected in
3.8		102	MB-1
5.2		208	MB-2
5.8		208	MB-1
6.1		265	MB-2
7.0		265	MB-2
7.5		342	MB-1
8.2		322	MB-2
9.2		322	MB-1 MB-2
9.5		322	MB-1
10.3		379	MB-2
12.1		428	MB-1
12.1		436	MB-1 MB-2
12.5		428	MB-1

Table 1. (Continued)

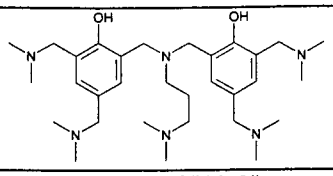
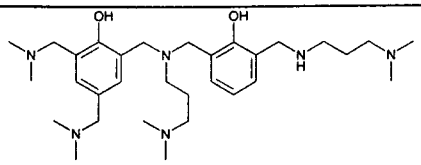
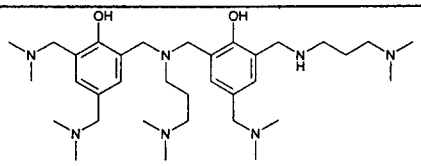
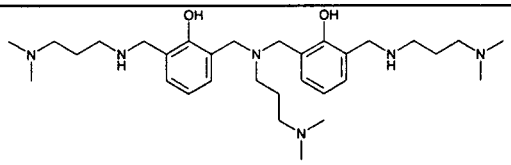
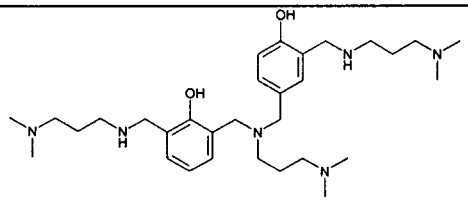
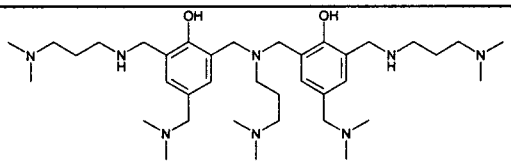
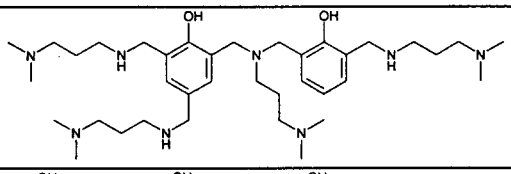
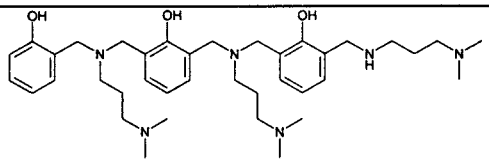
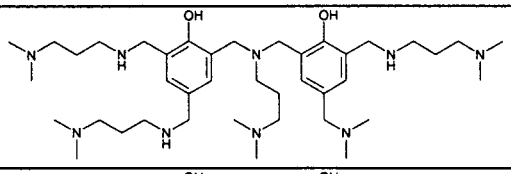
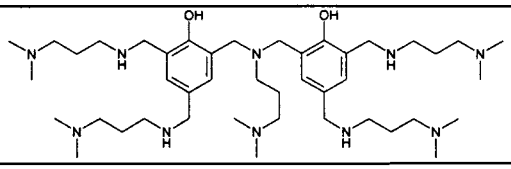
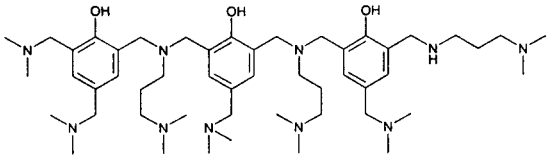
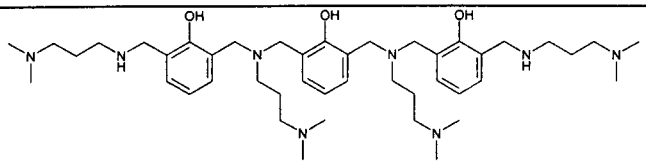
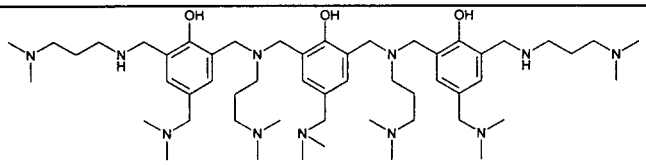
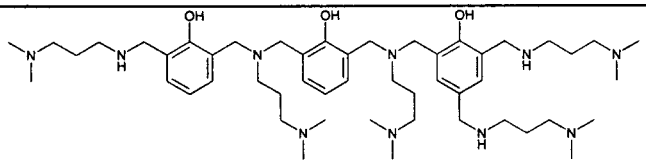
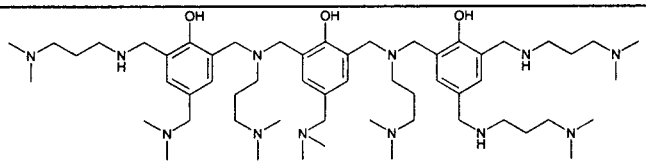
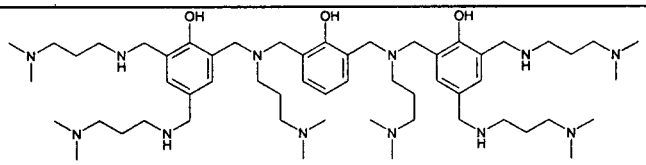
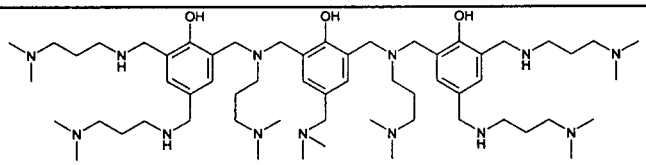
Retention time [min]	Structural proposal	Molecular weight [g/mol]	Detected in
12.6		542	MB-2
12.8	Isomer of "12.6"	542	MB-2
13.5		542	MB-2
13.7	Isomer of "13.5"	542	MB-2
13.9		599	MB-2
14.1	Isomer of "13.9"	599	MB-2
14.2		542	MB-1
14.6		542	MB-1
15.3		656	MB-2
15.8		656	MB-1
16.1		648	MB-1
16.2		713	MB-2
17.2		770	MB-1

Table 1. (Continued)

Retention time [min]	Structural proposal	Molecular weight [g/mol]	Detected in
17.2		876	MB-2
17.4		762	MB-1
18.0		933	MB-2
18.3		876	MB-1
18.5		990	MB-2
19.1		990	MB-1
19.2		1047	MB-2

[a] As far as possible, isomeric structures were identified by their mass spectra (formation of $[M + 13]^+$ and $[M + 25]^+$ ions in the case of ortho substitution with a secondary aminomethyl function) or by the plausibility of the formation of a certain substitution pattern of the phenol.

grams, see Figure 7), one prepared by the classical Mannich reaction (MB-1) and the other prepared by transamination (MB-2).

In addition to the typical mono-, di-, and trisubstituted phenols and residual free amine, MB-1 contains a relatively high amount of higher homologues formed by the Mannich reaction of a secondary aminomethyl function with a further phenol, a fact that also explains the higher viscosity compared to that of MB-2. A characteristic byproduct of Mannich bases made by the classical Mannich reaction is the trimerization product of the Mannich reagent (peak at 7.5 min). The *s*-hexahydrotriazine derivative (in this case 1,3,5-tris[3-(dimethyl-amino)propyl]-s-hexahydrotriazine [15875-13-5]) can be regarded as an indicator for the classical Mannich reaction. MB-2, in contrast, contains smaller amounts of higher molecular weight compounds. Large amounts of educt (peaks at 5.2 and 6.1 min) and partially transaminated Mannich

bases reveal the synthesis pathway via transamination. MB-2 contains only traces of residual free amine, a fact that might be important from a toxicological point of view. Process control and process optimization are facilitated by the possibility of determining the amounts of educt and partially transaminated byproducts quantitatively. SFC-UV, with absolute detection limits of about 50 ng for aliphatic amines (signal-to-noise ratio of 50 ng decylamine at 200 nm of 5:1) and 5 ng for phenolic Mannich bases may even be used for the quantification of traces of selected byproducts in phenolic Mannich bases.

CONCLUSION

Capillary SFC with dimethyl ether modified ethane as the mobile phase is an excellent technique for the separation and characterization of phenolic Mannich bases. SFC/MS allows the unambiguous identification of the constituents of such Mannich bases by their molecular weight and their fragmentation pattern, whereas UV detection allows the determination of the relative amounts of individual components. Qualitative and quantitative analysis of technically important phenolic Mannich bases is an important tool for process control and development as well as for the identification of unknown Mannich bases, where this method allows even a distinction between similar Mannich bases made via different synthetic pathways.

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