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Quantitative determination of native and methylated cyclodextrins by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Abstract

A method for the quantitative analysis of cyclodextrins using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry is described. To decouple the quantitative approach from the extreme dependence of the absolute ion signal on the experimental parameters of the MALDI technique, the method relies on the normalization of analyte molecular ion signals relative to internal reference molecular ion signals. For good crystal homogeneity and shot-to-shot reproducibility a new method of sample preparation using 4-hydroxy-α-cyanocinnamic acid as a matrix was developed. Linear calibration curves ($r^2 = 0.998$) were obtained for both permethylated cyclodextrins with homologous standards and β-cyclodextrin using maltohexaose as internal standard. Furthermore, tests on different 2,3- and 2,6-di-*O*-methylated cyclodextrins were made. An excellent shot-to-shot reproducibility allowed the analysis of byproducts using the molecular ion signal of the product as internal standard. In each instance, the results demonstrate that MALDI is a viable approach for quantification of low-molecular-mass analytes. © 1996 Elsevier Science Ltd.

Keywords: MALDI-TOF-MS; Quantification of cyclodextrins; Maltohexaose; Cyclomaltoheptaose (β-cyclodextrin); Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin

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1. Introduction

Introduced in 1987 by Hillenkamp and co-workers [1], matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry has become a powerful tool in the analysis of biomolecules and polymers. As a new soft ionization technique, MALDI enables the detection of nonvolatile high-molecular-mass molecules up to 200 kDa in the femtomol range without significant fragmentation. Since only nicotinic acid and 2,5-dihydroxybenzoic acid (DHB) [2] were used as matrix materials, several new matrices such as basic compounds [3] (pyridine derivatives) and cinnamic acid derivatives [4] were investigated to see whether they produce molecular ion signals of analytes. Mass determination of protein mixtures [5,6], oligosaccharides [7,8], underivatized DNA oligomers [9], oligonucleotides [10], and tryptic protein digests [11] are typical applications for MALDI. Most MALDI studies have focused on qualitative analysis.

Quantitative measurements are limited due to several problems associated with desorption and ionization from crystalline surfaces. The ion production process strongly depends on the laser irradiance, with the signal intensity increasing exponentially if the laser energy exceeds the desorption/ionization threshold [12]. Crystal inhomogeneity observed with several matrices (e.g. DHB) leads to poor shot-to-shot and sample-to-sample reproducibility. Therefore, variations in signal intensity occur when the laser beam moves across the sample. Vorm et al. [13] obtained more homogeneous surfaces using 4-hydroxy-α-cyanocinnamic acid as a matrix.

Recently, some other reports on quantitative aspects of MALDI have been published [14–19]. New methods of preparation using binary and ternary matrix mixtures as well as sample drying in high nitrogen flow have been described [14]. The matrix–comatrix complexes with DHB have shown better homogeneity of the crystal structure.

Much work has been done on quantitative determinations with MALDI employing internal standards. The use of nicotinic acid as matrix allows the determination of oligonucleotides [15]. A linear standard curve with tetramer (5′-AGTC-3′) as analyte and a dimer (5′-AG-3′) as internal standard was obtained. The absolute amount of cyclosporin A in blood was measured with cyclosporin D as an internal standard and DHB as a matrix [16], resulting in an accuracy of $\pm 10\%$. Another internal standard method for proteins (e.g. bovine insulin) developed by Nelson et al. [17] gave linear results over a concentration range covering an order of magnitude and achieved a relative standard deviation of 15%. This method using sinapinic acid (SA) as a matrix is suitable for large proteins like lactoferrin (molecular mass ca. 81 kDa). A correlation coefficient of 0.95 was calculated in experiments using an isotope-labelled 3,4-dihydroxy-phenylalanine (DOPA) standard for quantification of unlabelled DOPA [18]. Insufficient resolution of the TOF technique in higher mass ranges limits the application of isotope-labelled standards.

Only little work has been done on quantitative measurements of oligosaccharides. Harvey showed that good results could be obtained using internal and external standards for the analysis of complex oligosaccharides (molecular mass ca. 2 kDa) [19]. For oligosaccharides in DHB, signal intensity reflects sample quantity with no additional

standard, while enhanced linear correlation ($r^2 = 0.999$) was achieved using a fucosylated analogue of the carbohydrate as internal standard.

The internal standard spiking procedure seems to be more reliable, because it avoids the main problems mentioned above and is well suited for routine analysis. The use of standards with similar chemical properties [20] makes quantification less sensitive to variations in system parameters (primarily the laser power density).

There is still a need for new analytical methods to determine the relative amount of byproducts from methylated cyclodextrins used as chiral selectors in enantioselective capillary gas chromatography and capillary electrophoresis [21]. In the present paper, a fast preparation procedure with a 4-hydroxy-α-cyanocinnamic acid [13] matrix is reported for the quantification of native and methylated cyclodextrins.

2. Experimental

Materials.—4-Hydroxy-α-cyanocinnamic acid was purchased from Sigma Chem. Co. (C 2020), while LiCl was obtained from Merck (Darmstadt, Germany). α-Cyclodextrin (α-CD), β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), *O*-methyl-β-cyclodextrin (DS = 1.8) (Me-β-CD), were provided by Wacker Chemie (Munich, Germany). Hexakis(2,3,6-tri-*O*-methyl)-α-cyclodextrin (per-Me-α-CD), heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (per-Me-β-CD), and octakis(2,3,6-tri-*O*-methyl)-γ-cyclodextrin (per-Me-γ-CD) were purchased from Cyclolab (Cyclodextrin Research and Development Laboratory, Budapest, Hungary). Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (2,6-Me-β-CD) and heptakis(2,3-di-*O*-methyl)-β-cyclodextrin (2,3-Me-β-CD) were prepared in our research group as described in detail elsewhere [22].

The 2,6-Me-β-CD used for peak addition experiments was obtained from Fluka Chemika (Neu-Ulm, Germany). Maltopentaose, maltohexaose, and maltoheptaose were purchased from Merck (Darmstadt, Germany). Polymethylmethacrylate (PMMA) 1950 was obtained from Macherey-Nagel (Düren, Germany). Trifluoroacetic acid (TFA), ethanol and water were of HPLC grade, and obtained from Merck (Darmstadt, Germany). The other chemicals were of the highest purity commercially available. All chemicals were used without further purification.

Matrix-assisted laser desorption ionization mass spectrometry.—All data presented in this paper were obtained with a Bruker Biflex mass spectrometer. The spectrometer was equipped with a nitrogen laser of 337 nm and 5 ns pulse width as well as an ion reflector in the 1.7 m drift tube.

An acceleration voltage of 10 kV and the reflector positive ion mode (10.6 kV) were used to obtain mass spectra. Infinitely variable filters allowed the adjustment of laser power. The laser irradiance was not measured, but it was slightly above the threshold ($\approx 10^6$–10^7 W/cm^2). Detection in the reflected mode was achieved using a secondary electron multiplier (SEM) at a voltage of 2 kV. The detection signal was digitized at a sampling rate of 250 MHz and transferred to a workstation for storage and report generation.

Time-of-flight spectra were generated by signal averaging of 10 shots per point. This procedure was repeated nine times at different points, and the spectra were added to an

averaged spectrum of 100 shots. This procedure was used to enhance the signal-to-noise (S/N) ratio and to minimize possible errors due to crystal inhomogeneity and signal degradation (decreasing signals using the same point for several laser shots). Sample observation was enabled by an optical microscope (40 fold magnification). External calibration of the MALDI mass spectra was carried out with a second preparation using PMMA 1950 for calibration.

Analyte preparations for quantitative mass spectrometric investigations.—4-Hydroxy-α-cyanocinnamic acid was used as the matrix because signals were achieved at lower laser power than required with 2,5-dihydroxybenzoic acid. Saturated matrix solutions were prepared in ethanol at room temperature and centrifuged. In some experiments, LiCl (5 mg/mL) was added to the matrix solution. Stock solutions of the different dextrins, cyclodextrins and their derivatives (referred as carbohydrates below) were prepared by dissolving the carbohydrates in doubly distilled water to a concentration of 10 nmol/μL. Analyte solutions were made by serial dilution of stock solutions to a final concentration of 10 pmol/μL.

Good sample homogeneity was achieved by premixing the matrix and analyte solutions before deposition on the metal target. 45 μL of matrix solution were placed in an Eppendorf tube and solutions (2 × 5 or 3 × 5 μL) of different carbohydrates added in defined concentrations. In experiments performed with 2 aliquots of 5 μL carbohydrate solutions, additionally 5 μL of doubly distilled water were added for better crystallisation. The resulting mixture was stirred for a few minutes. The corrected absolute concentrations of carbohydrates in the matrix/analyte solutions are shown in Tables 1–3. Approximately 0.5 μL of the matrix/analyte solution was deposited on a polished stainless steel target and air-dried prior to its transfer into the source of the mass spectrometer. Every matrix/analyte solution was used to place eight samples on the target to average mass spectral data. All samples were examined with an optical microscope before being inserted into the vacuum system of the mass spectrometer. Usually the spots showed a porous, slightly yellow film.

Quantification experiments were performed using maltohexaose or per-Me-α-CD as the internal standard. In this case, 5 μL aliquots of 1 nmol/μL internal standard solutions were added to the matrix/analyte solution. In other experiments with homologous carbohydrates (like per-Me-α-CD, per-Me-β-CD, and per-Me-γ-CD) in different concentrations, one of the homologous compounds (e.g. per-Me-β-CD) was added as a 1 nmol/μL solution. This 1 nmol/μL carbohydrate was chosen as the internal standard (standard-per-Me-CD). For generation of reproducible mass spectra, the laser irradiation was adjusted to give a smooth baseline and a satisfactory S/N ratio. This lowest possible laser power is just above the ionization/desorption threshold, which is defined as the minimum laser irradiation necessary to produce mass spectra with a sufficient S/N ratio.

Evaluation of the spectra.—The baseline was corrected and subtracted in the mass-to-charge (m/z) region of the peaks needed for determination. The intensities of the analyte signals (I_{ana}) were normalized to that of the internal standard (I_{sta}). The procedures were the same for the evaluation of peak areas using the areas of the internal standard signals (A_{sta}) to normalize the areas of the analyte signals (A_{ana}). I_{ana}/I_{sta} (A_{ana}/A_{sta}) was used for normalization of the measured data, resulting in a direct

dependence of the ratio on the concentration of the analyte in the solution. I_{ana}/I_{sta} (A_{ana}/A_{sta}) reflects the ratio of ion yield between the analyte and standard. For every internal standard procedure, five 100 shot spectra were averaged. To compare the sample reproducibility (shot-to-shot) of spectra of equivalent preparations, the relative standard deviations (SD) were calculated. The average relative error was used to describe the relative SD achieved in quantification experiments.

3. Results and discussion

Samples were prepared and repeatedly measured to achieve a quantitative determination with MALDI-TOF-MS. First, the ion yields of equal concentrations of several homologous carbohydrates were compared. Up to 60 spectra were averaged and a relative error of 11.5% was calculated. The spectra obtained showed equal responses for all homologous analytes. For each compound, predominant molecular ion signals for the sodium and potassium adducts (MNa^+, MK^+) were present. Every spectrum generated had a constant ratio of intensities between the Na^+ and K^+ peaks. The Na^+ adduct signals were used for interpretation of the spectra.

Spectra obtained from comparative experiments with native cyclodextrins and modified cyclodextrins showed the expected difference in ion yield (spectra not shown). A constant relationship of intensities between different carbohydrate compounds appeared in experiments with β-cyclodextrin and maltohexaose (see below). This allowed quantitative determination of carbohydrates by means of an internal standard.

Some ternary mixtures of different concentrations of homologous permethylated cyclodextrins were prepared (see e.g. Fig. 1). The signal intensities of the analytes relative to the intensity of the 1 nmol/μL standard (I_{ana}/I_{sta}), as well as the SD and average relative error are listed in Table 1. A linear relationship between the normalized molecular ion peak intensities of analytes and concentrations of the analytes was observed ($r^2 = 0.9977$). A plot of the normalized intensities versus the concentrations is shown in Fig. 2. Below 100 pmol/μL and above 10 nmol/μL there was no linear correlation. Concentrations of analyte solutions less than 100 pmol/μL led to low absolute ion yields, while for highly concentrated preparations (e.g. 10 nmol/μL) solubility of the analyte in the matrix solution was poor. The results were strongly dependent on the preparation method and the solvents.

As mentioned above, there was a constant relationship in the response of native and modified cyclodextrins. The smallest errors (minimal rel. SD) were observed in comparision of some maltooligomers (degree of polymerization: 5, 6, 7) with native cyclodextrins. Based on these results, series of experiments were performed using the internal standard procedure. β-CD was used as the analyte, with concentrations varying between 100 pmol/μL and 8 nmol/μL, while maltohexaose was kept at a constant concentration of 1 nmol/μL (absolute concentrations of carbohydrates in matrix/analyte solution are shown in Table 2). Two time-of-flight spectra obtained with a binary mixture of β-CD and maltohexaose are presented in Fig. 3. Again a linear relationship ($r^2 = 0.9975$) between the normalized molecular ion signal intensities and the concentration of the

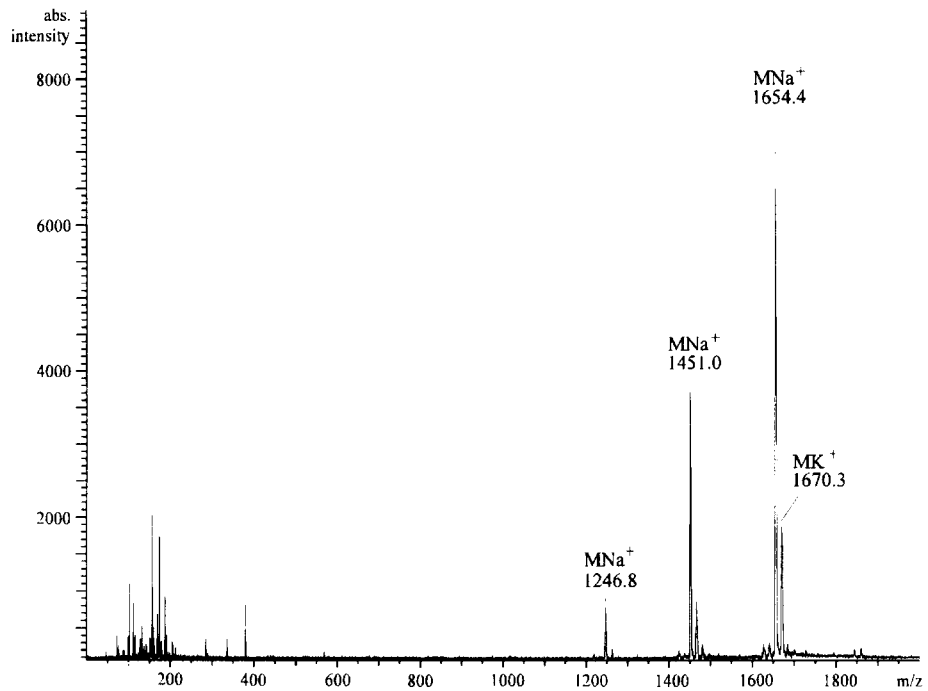

Fig. 1. 100 shot averaged MALDI-TOF spectrum from a ternary mixture of per-Me-α-CD, per-Me-β-CD, and per-Me-γ-CD. A matrix/analyte solution, containing 1 nmol/μL per-Me-α-CD (m/z 1222), 5 nmol/μL per-Me-β-CD (m/z 1428) and 10 nmol/μL per-Me-γ-CD (m/z 1632) analyte solutions was used to obtain the mass spectrum. In this mass spectrum per-Me-α-CD was chosen as the internal standard. Main peaks represent the Na^+ adduct signals ($\Delta m = 23$), while the K^+ adduct signals were observed at $\Delta m = 39$.

Table 1
Averaged normalized signal intensities (I_{ana}/I_{sta}) of per-Me-α-CD, per-Me-β-CD, and per-Me-γ-CD versus concentration of analyte. For each pair of analyte and standard the normalized signal intensities from 5–10 MALDI-TOF spectra were averaged. These results were obtained by preparing ternary mixtures of different concentrations of per-Me-α-CD, per-Me-β-CD, and per-Me-γ-CD. The compound, which was added as 1 nmol/μL solution to the matrix solution, was chosen as internal standard

Conc./type of analyte solution (nmol/μL)	Conc. of analyte in matrix/analyte solution (pmol/μL)	Conc./type of standard solution (nmol/μL)	I_{ana}/I_{sta} (average)	Relative SD	No. of averaged spectra
0.1/per-Me-α-CD	8.3	1/per-Me-β-CD	0.10	15	10
0.5/per-Me-β-CD	83	1/per-Me-γ-CD	0.55	4.2	5
1/–	250	1/–	1	0	5
3/per-Me-β-CD	416	1/per-Me-α-CD	3.11	7.1	5
5/per-Me-β-CD	583	1/per-Me-α-CD	5.35	4.3	5
7/per-Me-γ-CD	583	1/per-Me-α-CD	6.82	14.1	5
10/per-Me-γ-CD	833	1/per-Me-α-CD	10.8	9.6	5
Correlation			0.9977		
Av. rel. error			10.5%		

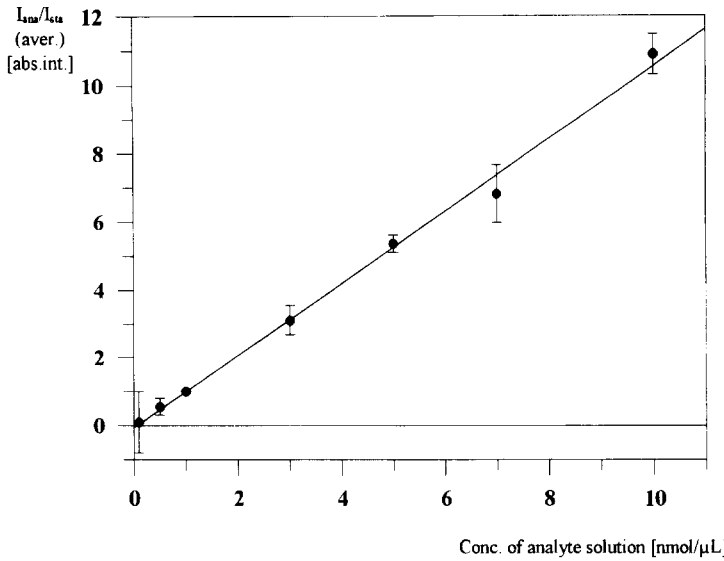

Fig. 2. Normalized intensity of per-Me-α-CD, per-Me-β-CD, and per-Me-γ-CD (intensity of per-Me-CD relative to the intensity of 1 nmol/μL standard per-Me-CD) versus concentration of per-Me-α-CD, per-Me-β-CD, and per-Me-γ-CD. Each point represents the averaged data of five 100-shot time-of-flight spectra with relative SD shown as error bars.

analyte was observed. An average relative error of 7.32% was calculated. All data obtained from these internal standard experiments are listed in Table 2 and Fig. 4.

The normalized analyte molecular ion signals increased linearly between 100

Table 2
β-CD signal intensities and peak areas normalized to a maltohexaose standard ($I_{\beta\text{-CD}}/I_{\text{maltohexaose}}$, $A_{\beta\text{-CD}}/A_{\text{maltohexaose}}$) versus concentration of β-CD. The β-CD signal intensities and peak areas were obtained from several sample preparations of β-CD in different concentrations with maltohexaose kept at a constant concentration of 1 nmol/μL. Each normalized β-CD signal intensity represents the averaged data from 4–5 MALDI-TOF spectra

Conc. of β-CD solution (nmol/μL)	Conc. of β-CD in matrix/analyte solution (pmol/μL)	Conc. of maltohexaose solution (nmol/μL)	$I_{\beta\text{-CD}}/I_{\text{maltohexaose}}$ (average)/relative SD	$A_{\beta\text{-CD}}/A_{\text{maltohexaose}}$ (average)/relative SD	No. of averaged spectra
0.1	8.3	1	0.24/2.4	0.22/6.8	5
1	83	1	1.50/3.5	1.90/6.3	5
3	250	1	4.3/6.3	5.55/7.4	5
5	416	1	6.6/11.1	8.28/7.4	5
7	583	1	9.95/11.5	12.5/13.9	4
8	583	1	10.8/9.1	13.4/11.6	4
(10)	833	1	10.7/9.5	13.8/15.7	4
Correlation			0.9975	0.9960	
Av. rel. error			7.32%	9.62%	

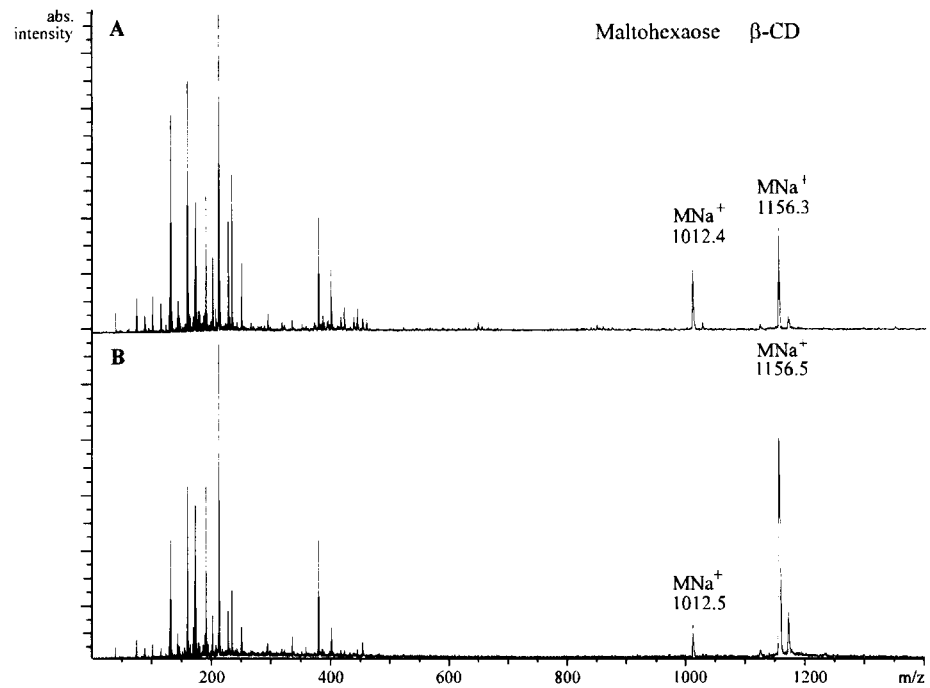

Fig. 3. Two mass spectra from the series of experiments using maltohexaose as internal standard and β-CD as analyte. The mixtures used contained maltohexaose solutions at a constant concentration of 1 nmol/μL, while β-CD solutions were added with concentrations of 1 nmol/μL (A) and 7 nmol/μL (B). 100 laser shots were averaged to produce both time-of-flight spectra.

pmol/μL and 8 nmol/μL with increasing β-CD concentration. A 10 nmol/μL β-CD solution added to the matrix solution produced molecular ion signal intensities similar to those of preparations with 8 nmol/μL β-CD solutions. As already mentioned, this can be explained by poor solubility of β-CD in the matrix/analyte solution. In solutions with a concentration of less than 100 pmol/μL, a poor S/N ratio was obtained, which did not allow spectra evaluation.

Calculation of quantitative data from the internal standard experiment were based on integrals instead of intensities (Table 2 shows the normalized integrated signal areas next to normalized intensities). The integral regions evaluated showed a linear relationship ($r^2 = 0.996$) and an average relative error of 9.62%. There were no major differences between the results of the integration procedure and the peak intensity data. This experiment indicates that there is no apparent advantage or disadvantage of using integrated signals.

Tests on modified cyclodextrins were also performed. The signal intensities of 2,3-Me-β-CD and 2,6-Me-β-CD (both at m/z 1352) were compared to a per-Me-α-CD standard. A concentration of 1 nmol/μL was used for the analyte and standard solutions. The spectra obtained showed an equal response of 2,3-Me-β-CD and per-Me-α-CD (aver. $I_{ana}/I_{sta} = 0.99$, SD = 0.04), while there was a minor difference in the peak

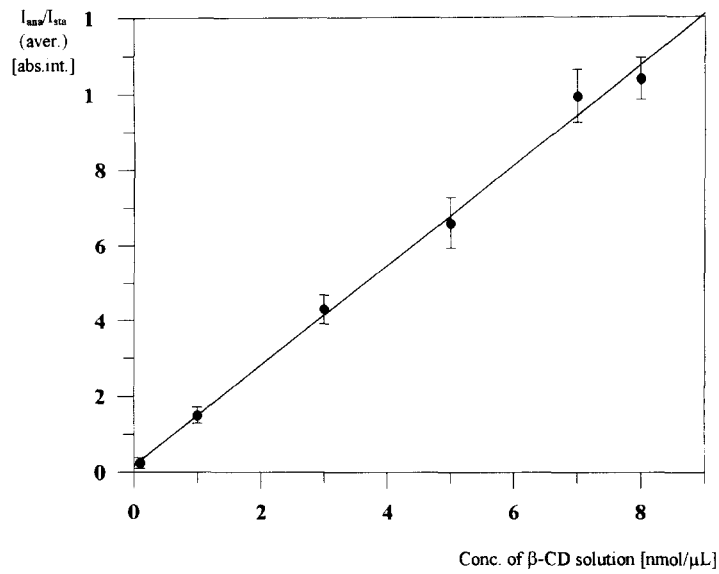

Fig. 4. β-CD intensity (normalized to maltohexaose intensity, derived from 1 nmol/μL standard solution) versus concentration of β-CD in analyte solution. Each point represents data averaged from five 100-shot time-of-flight spectra with relative SD shown as error bars.

intensities of 2,6-Me-β-CD and per-Me-α-CD (Fig. 5B). It should be pointed out that 2,6-Me-β-CD [prepared by our group, ≅ **(1)**] also contains a small portion of overmethylated material (peak at m/z 1366). Addition of the intensities of the main molecular ion signal and the signal of the overmethylated compound yielded the same response as for per-Me-α-CD (aver. $I_{ana}/I_{sta} = 1.02$, SD = 0.047).

Several other investigations were performed to prove that it was reasonable to add peak intensities. Per-Me-α-CD and 2,6-Me-β-CD (Fluka) solutions were prepared in the same manner as described above. The 2,6-Me-β-CD (Fluka) spectrum differed from that described above (Fig. 5A, B). Beside the main signal of the 2,6-methylated cyclodextrin (B), the spectrum shows a small peak of undermethylated compound (A), a large peak of overmethylated compound (C) and signals corresponding to the doubly (D) and triply (E) overmethylated cyclodextrin. Summing the peak heights of the species A–E gave the same value for the absolute intensitiy as that obtained for the per-Me-α-CD standard signal (aver. $I_{ana}/I_{sta} = 1.07$, SD = 0.044).

Me-β-CD (Wacker Chemie) and six different chromatographic fractions of 2,6-Me-β-CD together with under- and overmethylated byproducts were examined analogously (spectra not shown). After addition of the peak intensities of the product and byproduct signals, each mixture showed intensities comparable to that of the per-Me-α-CD-standard. The results of peak addition are presented in Table 3.

Because the peak heights for 2,3-Me-β-CD and per-Me-β-CD (diff. in methyl groups = 7) were comparable, equal responses of the different methylated cyclodextrins were assumed. Equal responses and good reproducibility of the spectra of methylated cyclodextrins allowed quantitative determination of under- and overmethylated bypro-

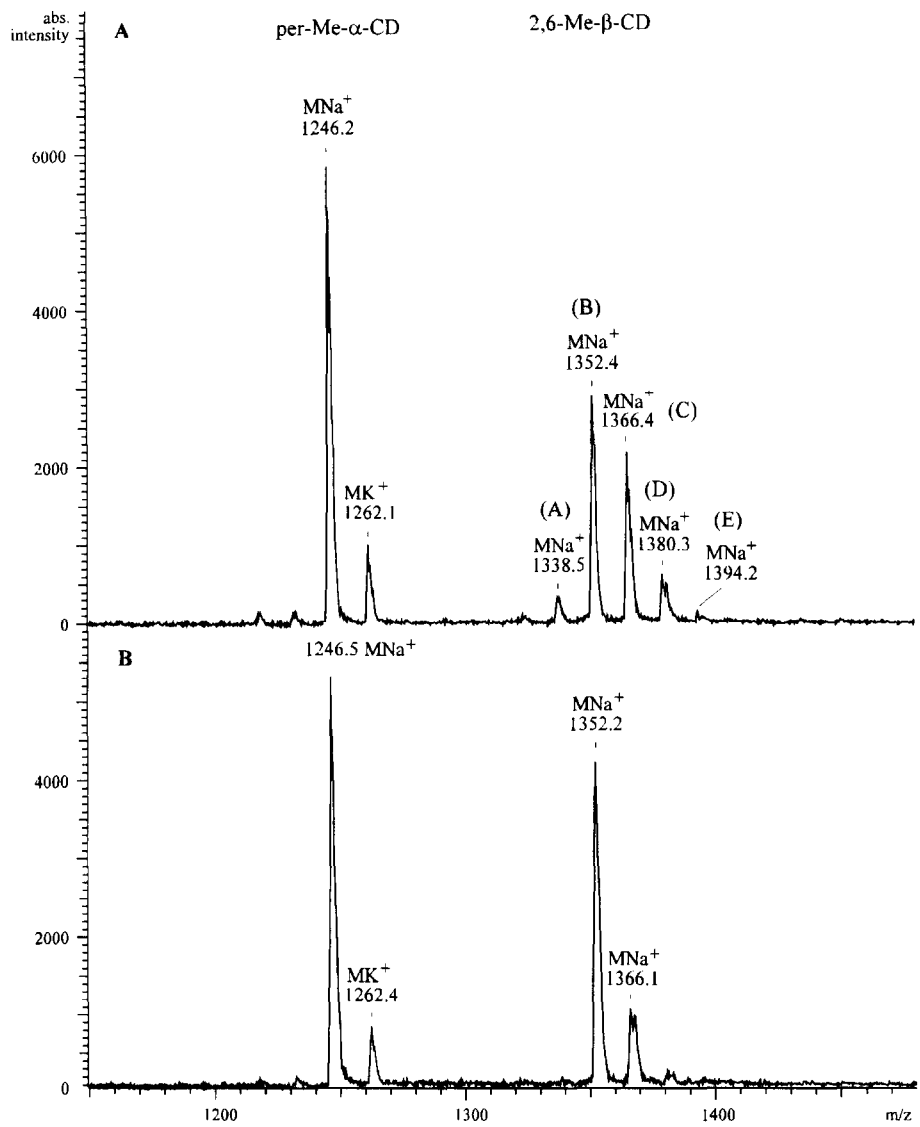

Fig. 5. Two mass spectra of an equimolar mixture (1 nmol/μL) of per-Me-α-CD and different samples of 2,6-Me-β-CD. The upper spectrum (A) shows the results for 2,6-Me-β-CD (Fluka Chemika), while (B) is a spectrum of 2,6-Me-β-CD, prepared in our group.

ducts relative to the main product signal at m/z 1352. Table 4 shows the results for quantification of byproducts. The intensities of byproduct peaks were correlated to the intensity of the main product peak (= 100). The calculated relative quantities permit an accurate description of the chemical purity of cyclodextrin derivatives. Using 2,6-Me-β-CD as a chiral selector for enantioselective capillary electrophoresis, Weseloh et al. [21] noticed a different separation behaviour depending on the amounts of byproducts. Thus,

Table 3
Sum of intensities and peak areas of several methylated cyclodextrins relative to the intensity and the area of a per-Me-α-CD-standard

Analyte	Conc. of analyte solution (nmol/μL)	Conc. of per-Me-α-CD solution (nmol/μL)	ΣI_{ana} / $I_{per\text{-}Me\text{-}\alpha\text{-}CD}$ (average)/ relative SD	ΣA_{ana} / $A_{per\text{-}Me\text{-}\alpha\text{-}CD}$ (average)/ relative SD	No. of averaged spectra
2,3-Me-β-CD	1	1	0.99/4.0	1.08/13.8	4
2,6-Me-β-CD (1)	1	1	1.02/4.6	1.15/11.3	5
2,6-Me-β-CD (Fluka)	1	1	1.07/4.1	1.16/1.3	4
Me-β-CD	1	1	0.99/8.5	1.06/10.4	5
2,6-Me-β-CD (chromatographic fraction 1)	1	1	0.98/3.3	1.05/6.7	4
(chromatographic fraction 2)	1	1	0.96/2.2	1.03/4.9	5

MALDI-TOF-MS can be a useful tool in the analysis of cyclodextrin based chiral selectors in enantioselective capillary gas chromatography and capillary electrophoresis [21].

All signals in the spectra of the peak addition experiments were integrated using special software. When peak areas were used instead of intensities for spectra evaluation, deviating results were obtained as shown in Table 3. The sum of analyte signal areas was by 5–10% larger than the area integrals of the standard signal. Peak broadening and insufficient resolution were responsible for the deviating results (see signal of the overmethylated compound M_2 in Fig. 6A). When these carbohydrates were analyzed, the K^+ adduct signal from the parent compound (M_1K^+ at m/z 1368, Δm to main Na^+ adduct signal = 16) and the Na^+ adduct signal of the overmethylated compound (M_2Na^+ at m/z 1366, Δm to main Na^+ signal = 14) could not be separated due to insufficient resolution. Using the integration method, the area of the K^+ adduct signal at m/z 1368 (M_1K^+) was added to the area of the M_2Na^+ signal of the overmethylated product.

Table 4
Content of byproducts of different preparations of 2,6-Me-β-CD. Peak intensities were used for evaluation of spectra and the obtained data were correlated to the response of the product peak (= 100). Each value represents the averaged data from 4–5 time-of-flight spectra

Analyte	1×Under-methylation/ relative SD	1×Over-methylation/ relative SD	2×Over-methylation/ relative SD	3×Over-methylation/ relative SD
2,6-Me-β-CD (1)	–	22.7/3.0	–	–
2,6-Me-β-CD (Fluka)	–	78.9/3.8	23.6/5.2	–
2,6-Me-β-CD chromatographic fraction 1	–	22.5/17.7	82.4/11.3	20.3/7.5
chromatographic fraction 2	321.4/6.3	49.1/6.3	24.7/7.7	–

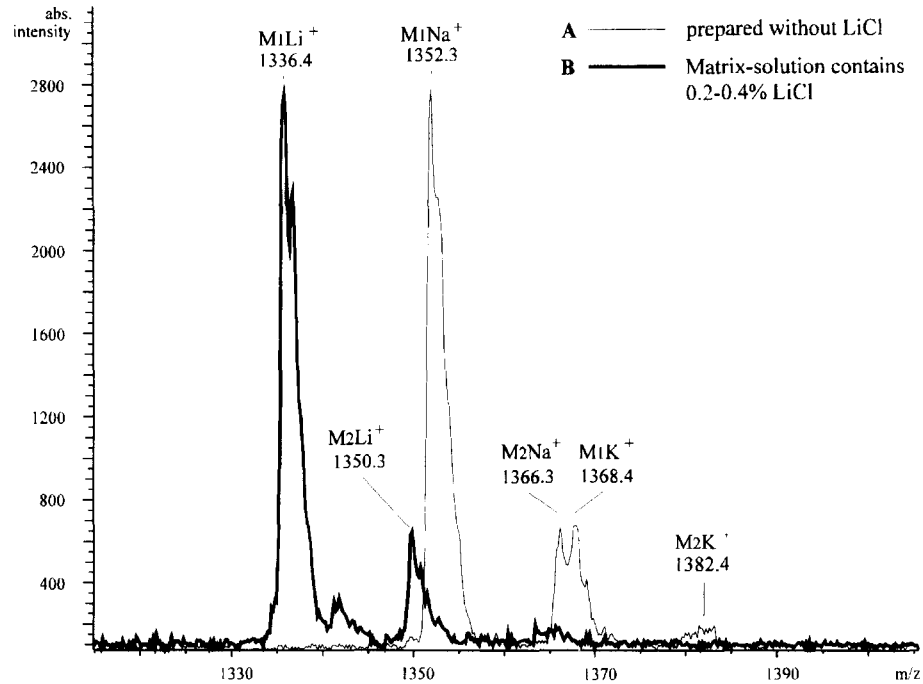

Fig. 6. Two time-of-flight spectra of 2,6-Me-β-CD (synthesized in our group) obtained by the conventional preparation method (A) and by a modified procedure using matrix solutions containing 0.2–0.4% LiCl (B).

To suppress K^+ adduct peaks, a modified sample preparation was performed. LiCl (0.2–0.4%) was added to the matrix solution before completing the matrix/analyte solution. A matrix/analyte solution was prepared composed of 1 nmol/μL per-Me-α-CD as the standard and 1 nmol/μL 2,6-Me-β-CD (**1**) as the analyte in a matrix solution containing LiCl (Fig. 6B). Five spectra of this solution were generated and an average relative error of 11.2% was observed. The spectrum shown contains only Li^+ adduct peaks. Neither Na^+ nor K^+ adduct signals were observed, and the peaks of overmethylated compound are narrower because of the absence of unresolved K^+ adduct signals. Spectra from both preparation techniques gave identical ratios of peak heights of the overmethylated product to those of the parent molecular ion signals (Fig. 6A, B). The spectra show equal peak intensities for both overmethylated products. This indicates that there were no additive effects in the intensities of the unresolved K^+ adduct peak and that of the overmethylated compound. When samples were prepared without LiCl, the response of the badly resolved overmethylated compound (m/z 1366) was not influenced by the K^+ adduct signal at m/z 1368.

Several tests were performed using the LiCl-containing matrix solution. In some experiments the modified procedure did not give reproducible results because the crystallization process was influenced by salt contamination.

In the quantification experiments with methylated cyclodextrins presented above, better results could be achieved using samples without additional LiCl for the prepara-

tion. Using intensities instead of areas for evaluation and interpretation, yielded the best results.

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