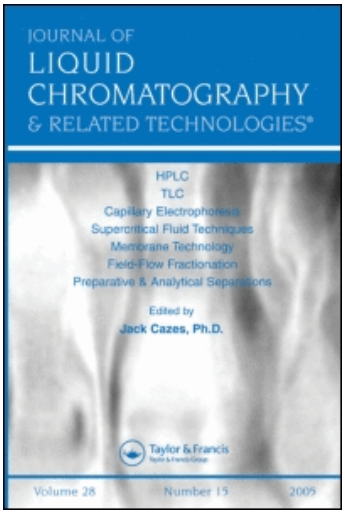

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ADVANTAGES OF EVAPORATIVE LIGHT SCATTERING DETECTION FOR THE PURITY CONTROL OF COMMERCIAL CYCLODEXTRINS

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ABSTRACT

Using an Evaporative Light Scattering Detector (ELSD) which is a universal detection system compatible with gradient elution, allowed satisfactory analysis of the non volatile impurities in cyclodextrins. ELSD performances for cyclodextrin analysis were evaluated. The detection capabilities of ELSD are better than those of RI and Polarimetric detectors (detection thresholds obtained with the ELSD are about 80 ng). Analyses were performed on different Phenyl columns with acetonitrile-water mobile phases in isocratic or gradient mode. Potential impurities of β-CD and of TM-β-CD, which included 7 linear (glucose to maltoheptaose), 2 unbranched cyclic (α- and γ-CD) glucose polymers and partially methylated cyclodextrins, have been determined.

As the ELSD response factors of these homologous compounds are highly similar, it is possible to evaluate impurity levels without previous identification. The methods presented provide good selectivity and sensitivity and can also be used to compare different sources of cyclodextrins.

INTRODUCTION

β-cyclodextrin (β-CD) and methylated β-cyclodextrins are used in various fields, such as in the manufacture of cosmetics, food technology, the pharmaceutical industry and in the production of agrochemicals, for their ability to include different sized guest molecules.[1] However, the analysis as well as the isolation in high purity of these cyclodextrins are still a real problem.

Commercially, β-CD is produced from the enzymatic digestion of starch.[2] The β-CD is selectively purified from the crude digest containing compounds with highly different polarities like glucose, linear glucose polymers, cyclic hexamer (α-CD), cyclic octamer (γ-CD) and cyclic heptamer (β-CD). An analytical method was needed to determine the level of potential linear and unbranched cyclic polymeric glucose impurities in β-CD.

β-CD has often been modified chemically. Methylation of β-CD to form 2, 6 or 2, 3, 6-tri-O-methyl derivatives[3] significantly improves aqueous solubility and enhances complexation ability.[4] However, the preparation of defined methyl derivatives of β-CD is not without chemical problems related to the difference in the reactivity of the hydroxyl groups at positions 2, 3 and 6 of cyclodextrin rings.[5] It is therefore necessary to be able to follow the progress of the reaction, to calculate the reaction yield and to characterise the obtained products.

Various high performance liquid chromatographic (HPLC) methods have been applied for maltodextrin and cyclodextrin analysis using such columns as aminopropyl-bonded,[6-11] aminocyano-bonded,[12] C_8 and C_{18}-bonded ,[7,11,13-17] and ion exchange columns.[18-21] More recently the use of graphitized carbon columns has been reported.[22]

The main difficulties during the analysis of carbohydrates are caused by the limitations of the detection systems, due to the absence of UV chromophore groups in cyclodextrins and maltodextrins. Several means of detection have been employed including refractive index,[6,7,9,11-13,16,19,22] pulsed amperometric,[21,23] fluorescence,[24] polarimetric [9,11] and indirect photometric detection.[25]

Both refractive index detection and polarimetric detection are very sensitive to changes in the refractive index of the mobile phase caused by variations in temperature, pressure and mobile phase composition.[26] As a result, the lack of sensitivity of these detection modes and the impossibility of carrying out gradient elutions with these detectors on one hand and the complexity of the derivatisation systems on the other, have given rise to the development of light scattering detection.[10,20,27]

In fact, the evaporative light scattering detector (ELSD) which, like refractometry, is a universal detector, is even more sensitive,[28] easy to use (because of the absence of base-line drift and its rapid equilibration), and compatible with gradient elution. It has also been shown to be a good choice for analytical studies of carbohydrates [29] and more recently for cyclodextrin analysis.[27]

In this paper, ELSD performances for cyclodextrin analysis are described. The limits of detection, response factors and quantitative impurity results obtained are reported. Different isocratic and gradient LC systems have been optimized to control the purity of commercial β-CD and TM-β-CD.

EXPERIMENTAL

Apparatus

The liquid chromatographic apparatus consisted of a Varian (Palo Alto, CA, USA) Model 9010 gradient pump, a Rheodyne (Berkeley, CA, USA) Model 7125 injector with a 20-µl sample loop and two types of detectors: an Evaporative Light Scattering Detector (ELSD) (Sedere, Alfortville, France) Model Sedex 55 and a Refractive Index Detector (Showa Denko, Tokyo, Japan) Model Shodex RI-71.

The usual ELSD detector settings were as follows: photomultiplier, 9; evaporative temperature, 30°C; air pressure, 2.2 bar. Data were processed using a Shimadzu (Kyoto, Japan) Model CR 5A integrator.

The following columns were used: Zorbax Phenyl (250 x 4.6 mm I.D.) (Rockland Technologies, INC., Newport, DE, USA) and Nucleosil Phenyl (150 x 4.6 mm I.D.) (Macherey-Nagel, Düren, Germany). The flow rate was 1 mL/min, experiments were carried out at room temperature.

Reagents

Acetonitrile (RS for LC) was purchased from Carlo Erba (Milan, Italy) and water from the Elgastat UHQ II System from Elga (Antony, France).

Glucose and maltose were obtained from Merck (Darmstadt, Germany), heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD), maltotetraose, maltopentaose, maltohexaose and maltoheptaose from Sigma (S^t Louis, MO, USA), α-, β-, γ-CD from Wacker (Lyon, France), heptakis (2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) from our laboratory.

The sample solvent for maltodextrins and α-, β-, γ-CD was pure water and 50:50 acetonitrile-water for DM-β-CD and TM-β-CD.

RESULTS AND DISCUSSION

ELSD Performances for Cyclodextrin Analysis

ELSD performances for cyclodextrin analysis have been studied in order to optimize the cyclodextrin response, to compare the responses of cyclodextrins and to obtain a crude estimation of the linearity of the detection system and an approximation of the detection threshold.

The principle of the Evaporative Light Scattering Detector (ELSD) is to nebulize the column effluent into droplets which are carried by a nebulizing gas (air, nitrogen, etc.) into an evaporator tube and then directed towards a light beam. Light is scattered by residual particles of non-volatile analyte and measured by a photomultiplier, providing the chromatographic signal.[30]

Column effluent nebulization favours the elimination of the solvent constituting the mobile phase while avoiding partial vaporization of the solute. Increasing the evaporator tube temperature causes solutes which possess a moderate or high molar volatility to evaporate and, consequently, the scattered light has a lower intensity.

Detector Response

Table 1 reports peak-areas of cyclodextrins and glucose according to the temperature of the detector evaporator tube.

Table 1

Peak-Area of α-, β-, γ-CD, TM-β-CD and Glucose According to the Temperature of the Detector Evaporator Tube

		Evaporator Tube Temperature			$\frac{\Delta A^*}{A_{30}}$
		30°C	50°C	80°C	
Glucose	tr_{min}	1.88			
	Peak-area	6 100 256	5 358 328	4 715 938	23 %
α-CD	tr_{min}	2.21			
	Peak-area	6 784 958	6 639 871	6 038 746	11%
γ-CD	tr_{min}	4.44			
	Peak-area	5 424 940	5 188 351	4 869 416	10 %
β-CD	tr_{min}	6.20			
	Peak-area	4 166 424	4 148 751	3 955 971	5 %
TM-β-CD	tr_{min}	8.60			
	Peak-area	8 207 192	6 576 868	6 083 077	26 %

* $\frac{\Delta A}{A_{30}} = \frac{A_{30} - A_{80}}{A_{30}}$ x 100; A_{30}, A_{80} are the peak-area values for each compound at 30°C and 80°C.

Column, Nucleosil Phenyl (150 x 4.6 mm I.D.); mobile phase, 100 % water for glucose, acetonitrile-water (5:95) for α-, β-, γ-CD, acetonitrile-water (40:60) for TM-β-CD; flow-rate, 1 mL/min; concentration of each solute, 500 mg/L.

The composition of the injection solvent and of the mobile phase was the same; ELSD Sedex 55.PM = 9, P = 2.2 bar.

Whatever the retention of solutes (near the void volume or with a great retention), we noted that the response of cyclodextrins, like that of glucose, decreased with increasing temperature from 30°C to 80°C. The best detector response is with a vaporization tube temperature of 30°C.

TM-β-CD, like glucose, is subject to a higher variation of peak-area according to the temperature, than the native cyclodextrins (α-, β- and γ-CD). Values of $\frac{\Delta A}{A_{30}}$ clearly depict this result.

For all studied solutes, the best detector response is obtained with a vaporization tube temperature equal to 30°C. This result is in good agreement with that already published for sugars, i.e. a low evaporator tube temperature is better than a high temperature.[31]

For given detector parameters, TM-β-CD like α-, β- and γ-CD, has a better response than glucose. For a given mobile phase composition (acetonitrile-water, 5:95), for α-, β- and γ-CD, the more the compound is retained on the stationary phase, the lower the peak-area is. However, when these same solutes are eluted near the void volume by increasing the acetonitrile percentage in mobile phase, their detector response is nearly equal. So, these differences seem to depend on the detection system rather than on the nature of the solute. The evaporation yield decreases when the solute retention increases. These results are in good agreement with those commonly obtained with series of homologous compounds.[32,33]

For a sample concentration of 500 mg/L, TM-β-CD has a higher detector response than β-CD although its retention time was greater than that of β-CD (Table 1). Two reasons can explain this fact: firstly, elution of TM-β-CD requires a greater amount of acetonitrile (40 %) in the eluent than elution of β-CD (5 %). Therefore, the mobile phase is easier to evaporate in the case of TM-β-CD elution. Consequently, nebulization yield is better (the more the organic solvent percentage increases in the mobile phase, the more the nebulization yield increases) and the size of droplets may be lower.[30] So, detector sensitivity is better for TM-β-CD than for α-, β-, γ-CD. Secondly, the methylation of all hydroxyl groups of β-CD to obtain TM-β-CD has modified the physical properties of β-CD. This is confirmed by the fact that, when β-CD and TM-β-CD are eluted near the void volume with the same mobile phase, the TM-β-CD response is higher than the β-CD one.

Calibration Curves

In order to achieve accurate quantitative analysis of α-, β-, γ-CD and TM-β-CD, calibration curves have been carried out with water-acetonitrile eluent on a Nucleosil Phenyl column in an isocratic mode. Although the variation of

Table 2

Calibration Curves Log A* = b Log C* + Log a for α-, β-, γ-CD, and TM-β-CD Studied Concentration Range: 2.5 to 1000 mg/L

Solute	b*	Log a*	Correlation Coefficient	Retention Time (min)
α-CD	1.38	6.98	0.9994	2.2
γ-CD	1.40	6.70	0.9994	4.3
β-CD	1.42	6.33	0.9994	6
Average value	1.40	6.67	0.9994	---
R.S.D. (%)	1.43	4.89	---	---
TM-β-CD	1.67	5.35	0.9993	8.7

*Where A is the measured peak-area, C is the cyclodextrin concentration, b is the slope, and Log a is the γ-intercept.
Column, mobile phase and detection as in Table 1.

the ELSD response is complex (it depends on droplet size, concentration and nature of solute, gas and liquid flow rates, vaporization temperature, etc.), it was assumed that in a large range of sample size the measured peak area can be related to sample size by the following relationship:[34]

$$A = a\,C^{b}$$

Where b is the slope of the response line, C is the solute concentration and a is the response factor. As a result, the linearity between surface area response and concentration is obtained in double logarithmic coordinates.[35] (Curve parameters are reported in Table 2.).

$$\mathrm{Log}\,A = b\,\mathrm{Log}\,C + \mathrm{Log}\,a$$

For the four cyclodextrins studied, graphs were linear with an acceptable correlation coefficient ($R \approx 0.9994$). This result was in agreement with the quantitative determination of sugars.[29] The linear dynamic range is almost 3 decades (concentrations vary from 2.5 to 1000 mg/L).

Slope b mentioned in the literature has values generally comprised between 1 and 1.6, with 1.3 being the most representative value.[29] Slopes obtained for α-, β- and γ-CD are contained in the expected interval and are close to the most representative value 1.3 and also to those of sugars, for which the slope is 1.24 [29] (average slope for α-, β- and γ-CD is 1.40).

It has been observed by different authors that within a group of homologous compounds, the detector response is nearly equal.[34,35] Considering the low value of the relative standard deviation of slope b (R.S.D. = 1.43 %) and of the intercept Log a (R.S.D. = 4.89 %), the detector response can be considered as equal for α-, β- and γ-CD. On the other hand, for TM-β-CD, the slope is slightly higher (b = 1.67) and the intercept is slightly lower (Log a = 5.35) than those of α-, β- and γ-CD. These variations of curve parameters seem due, like variations on peak-area, to the eluent composition (richer in acetonitrile for TM-β-CD elution than for α-, β- and γ-CDs elution) and to modifications of TM-β-CD physical properties.

We concluded that, in order to achieve a more accurate determination of impurities, it was necessary to make one calibration curve specially for TM-β-CD.

Repeatability

The repeatability of the analysis which is expressed by the relative standard deviation of the area of each cyclodextrin peak has been also evaluated (Table 3). The repeatability determined from five replicate injections was satisfactory for all the cyclodextrins studied (α-, β-, γ-CD and TM-β-CD): the deviations varies only from 1.14 % to 3.58 %.

Detection Limits

Table 4 lists detection limits for α-, β-, γ-CD and TM-β-CD obtained with the RI and with the ELSD. Using the most sensitive range of the ELSD, a 50 ng (20 µl of 2.5 mg/L) detection limit of α-cyclodextrin and 80 ng (20 µL of

Table 3

Repeatability for Standard Solutions of α-, β-, γ-CD, and TM-β-CD

Solute	α-CD (350mg/L)	β-CD (350 mg/L)	γ-CD (500 mg/L)	TM-β-CD (300 mg/L)
Peak-area	4 166 611	4 339 606	5 452 070	3 881 604
	4 155 605	4 381 030	5 366 712	4 057 117
	4 019 240	4 271 929	5 305 771	3 970 371
	3 957 053	4 248 159	5 330 156	3 987 729
	4 218 230	4 198 303	5 423 002	3 693 827
Mean	4 103 348	4 287 805	5 375 542	3 918 130
R. S. D. (%)	2.68	1.70	1.14	3.58

Column, mobile phase and detection as in Table 1.

Table 4

Comparative Detection Limits for α-, β-, γ-CD, and TM-β-CD with a RI and an ELS Detector

	ELS Detector	RI Detector
Solute	**Detectable mass on column*(ng)**	
α-CD	50	2 000
β-CD	80	2 000
γ-CD	80	2 000
TM-β-CD	120	1 000

*When signal-to-noise ratio (S/N) equals 3.
Column, Nucleosil Phenyl (150 x 4.6 mm I.D.); mobile phase, acetonitrile-water (5:95) for α-, β- and γ-CD, acetonitrile-water (40:60) for TM-β-CD; compounds are in solution in the eluent.

4 mg/L) detection limit of β- and γ-cyclodextrins are obtainable, at a signal-to-noise ratio of 3. So, it is seen that the minimal detectable quantity achieved by the ELSD is much lower than that of the RI detector (2 000 ng for α, β, γ-CD and 1 000 ng for TM-β-CD with the RI detector). The detection thresholds we have obtained with the Shodex RI-71 detector were comparable to those obtained with another RI detector previously published by G. White et al.[12] The minimal detectable quantity achieved with the polarimetric detector is also higher than that of the ELSD.

Indeed, the detection limit obtained for TM-β-CD with the polarimetric detector was 2000 ng.[11] So, the level of sensitivity of the ELSD was suitable to provide a satisfactory impurity profile of cyclodextrins.

We can conclude that the ELSD is suitable for performing the assay of impurities in cyclodextrins to ensure quality in these products.

Analysis of a Commercial β-CD

The β-CD is selectively purified from the crude digest of starch containing linear glucose polymers (glucose to maltoheptaose), α-CD and γ-CD. So, an analytical method allowing the separation of these compounds, characterized by very different polarities, was necessary. Moreover, to obtain the best of all possible sensitivities, the analytical method has to permit elution of all potential impurities before the major product.

The separation of linear glucose polymers and cyclodextrins has already been studied on amino [7,8] and amino-cyano [12] columns with acetonitrile-water as mobile phase. Detection is commonly performed by RID, so analyses must be performed isocratically. In these chromatographic conditions, cyclodextrins are eluted with elution times comparable to those of maltooligosaccharides. Besides, the overlapping of cyclodextrin and maltodextrin chromatographic peaks prevents the determination of small amounts of impurities in the presence of a large proportion of β-CD and leads to a reduction in sensitivity.

On the other hand, cyclodextrins are strongly adsorbed on octadecyl bonded silica column.[29] So, on this type of support, the percentage of organic modifier in the aqueous-acetonitrile mobile phase necessary to elute these compounds was so high that linear sugars were eluted near the void volume in these conditions.

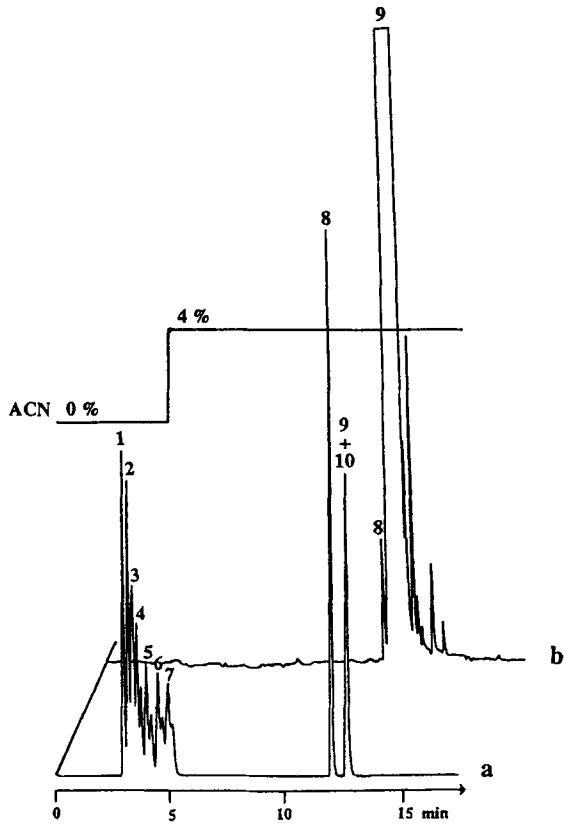

Figure 1. (a) Analysis of a standard mixture of β-cyclodextrin and nine potential impurities. (b) Analysis of a commercial β-cyclodextrin (10 000 mg/L). Column: Zorbax Phenyl (250 x 4.6 mm I.D.); gradient elution, water during 5 min, then acetonitrile-water (4:96) during 10 min ; flow-rate, 1 mL/min ; evaporative light scattering detector.
Elution order: 1, glucose; 2, maltose; 3, maltotriose; 4, maltotetraose; 5, maltopentaose; 6, maltohexaose; 7, maltoheptaose; 8, α-CD; 9, β-CD; 10, γ-CD.

With a lower acetonitrile percentage, good separation of malto-oligosaccharides was observed but cyclodextrins are not eluted. Simultaneous analysis of cyclodextrins and maltodextrins on octadecyl bonded silica column requires gradient elution and the use of a detector compatible with this elution mode.

Our previous study, concerning the RP-LC analysis of methylated β-CD[27] has showed that phenyl bonded silica column offers selectivities that are different from those obtained using classical C_{18}-bonded silica column and allows to achieve satisfactory isocratic analysis of these compounds with a shorter time.

In this paper, Fig. 1a shows the separation of a standard mixture of β-CD and of its potential impurities which included 7 linear (glucose to maltoheptaose) and 2 unbranched cyclic (α- and γ-CD) glucose polymers. The compounds are detected by ELSD, following separation on a Zorbax phenyl column with an acetonitrile-water gradient. This gradient consists in 100 % of water during 5 min, then step gradient to acetonitrile-water (4:96) during 10 min.

An entirely aqueous medium was necessary to retain and to separate glucose to maltoheptaose. Elution occurs in order of increasing molecular mass. Despite their short elution times (tr ≤ 5 min), the α- and β-anomers of maltotetraose, maltopentaose, maltohexaose and maltoheptaose are resolved. On the other hand, a small amount of acetonitrile was necessary to elute cyclodextrins which show more hydrophobic interaction than linear sugars. So it is easier, than on amino columns, to separate the cyclic glucooligomers from the linear glucooligomers.[12] Consequently, phenyl bonded-silica column with gradient elution provided an efficient means of isolating cyclodextrins from maltooligosaccharides because of the great difference of retention of these compounds. In addition, the use of gradient elution allows a rapid separation of the compounds (<13 min).

To show the potential of this new method, a commercial sample of β-CD (10 000 mg/L in 100 % water) was analyzed in these chromatographic conditions (Fig. 1b). The commercial β-CD did not exhibit any of the linear glucose impurities. At the end of the analysis, by increasing the percentage of acetonitrile to 20 %, we ascertained the absence of other non-eluted impurities in the chromatographic conditions of the standard mixture analysis. However, the batch contained a small amount of α-CD and of other impurities which are eluted after β-CD.

This system did not permit the γ-CD chromatographic peak to show because β- and γ-CD are not resolved in these conditions. So another chromatographic system has been studied in order to improve separation between β- and γ-CD.

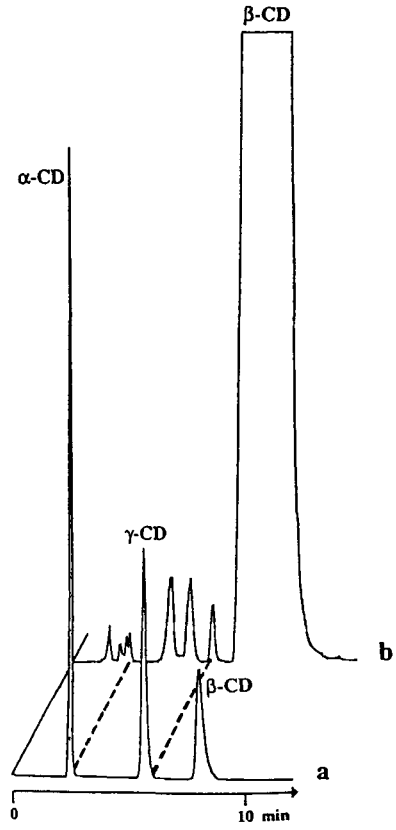

Figure 2. (a) Analysis of a standard mixture of α-, β-, and γ-cyclodextrin. (b) Analysis of a commercial β-cyclodextrin (10 000 mg/l). Column, Nucleosil Phenyl (150 x 4.6 mm I.D.); eluent, acetonitrile-water (4:96) (v/v); flow-rate, 1 mL/min; evaporative light scattering detector.

Fig. 2a depicts a chromatogram illustrating the high resolution ($R_s \geq 4$) and rapid separation (< 9 min) of α-, β- and γ-CD on a Nucleosil phenyl column in isocratic mode with acetonitrile-water (4:96) as eluent. On a Nucleosil phenyl column, the elution order is the same as on C_{18}-bonded silica columns:[11,17] β-CD is more retained than γ-CD which is more retained than α-CD. But the advantage of this system with regard to C_{18} columns is that the chromatographic analysis is shorter and efficiencies are better.

On Phenyl bonded silica column (Nucleosil or Zorbax), acetonitrile-water mixtures were found to be very suitable solvents for developing the chromatograms of cyclodextrins, whereas on C_8 or C_{18}-columns, aqueous methanolic or ethanolic solutions provide better results than acetonitrile-water mixtures.[11]

The commercial β-CD (10 000 mg/L) has also been analyzed in these chromatographic conditions. Fig. 2b shows that the sample of β-CD did indeed contain a small amount of α-CD but also a small amount of γ-CD. We could also detect two other unidentified impurities which are eluted between α- and γ-CD (in the previous system, they were eluted with the peak of β-CD). These results prove that the two systems are complementary: with Zorbax Phenyl, characterization of linear sugars traces and of impurities which are more hydrophobic than β-CD is possible, whereas linear sugars are eluted near the void volume without separation on Nucleosil Phenyl even if 100 % water is used as mobile phase. With Nucleosil Phenyl, the high selectivity between α- and β-CD allows detection of γ-CD traces which are eluted with the same retention as β-CD on Zorbax Phenyl.

Calibration curves previously established have allowed the quantification of the proportion of α- and γ-CD in β-CD. The commercial β-CD contains about 0.05 % (w/w) of α-CD and about 0.2 % (w/w) of γ-CD. Considering all of the impurities and the fact that they have an ELSD response close to that of β-CD, the degree of β-CD purity could be estimated at 99.3 %.

So Nucleosil phenyl column with acetonitrile-water mobile phase is a suitable system to quantify small amounts of α- and γ-CD in the presence of a large proportion of β-CD.

Analysis of a Commercial TM-β-CD

A similar study has been carried out to determine the potential impurities of TM-β-CD in which the three OH groups of every glucose unit are replaced by an $O\text{-}CH_3$ group. In view of the complexity of β-CD alkylation methods, an analytical method was needed in order to determine the degree of purity of commercial and synthetic samples.

Investigations into the analysis of methylated and partially methylated cyclodextrins by HPLC have already been reported.[24,36-39] The methyl derivatives of β-CD are much more strongly adsorbed on hydrophobic

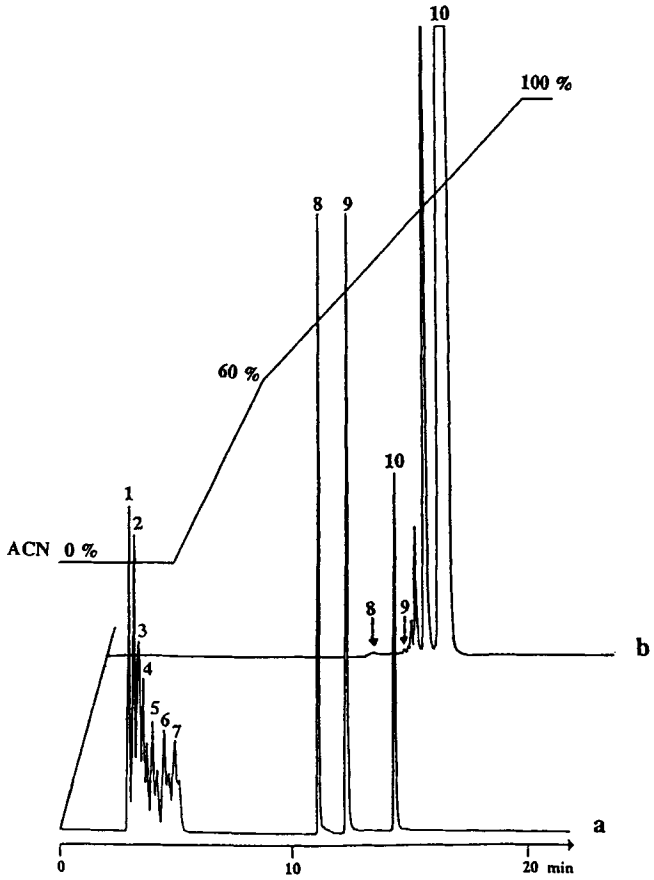

Figure 3. (a) Analysis of a standard mixture of TM-β-CD and nine potential impurities. (b) Analysis of a commercial TM-β-CD (10 000 mg/l). Column, Zorbax Phenyl (250 x 4.6 mm I.D.); eluent, acetonitrile-water : water during 5 min, followed by gradient elution from 0 % to 60 % acetonitrile in 4 min, then to 100 % acetonitrile in 11 min; flow-rate, 1 mL/min; evaporative light scattering detector.
Elution order: 1, glucose; 2, maltose; 3, maltotriose; 4, maltotetraos; 5, maltopentaose; 6, maltohexaose; 7, maltoheptaose; 8, β-CD; 9, DM-β-CD; 10, TM-β-CD.

stationary phases than the parent β-CD itself. Their elution requires higher concentrations of organic additive in the aqueous mobile phase solutions. So, simultaneous analysis of compounds with such widely differing polarities as linear sugars, β-CD and methylated β-cyclodextrins requires gradient elution which precluded the use of an RI detector.

Figure 3a presents the chromatogram of a standard mixture of β-CD, DM-β-CD, TM-β-CD and also linear sugars (glucose to maltoheptaose). These compounds are detected by ELSD after being separated on a Zorbax Phenyl column with an acetonitrile-water gradient. Mobile phase consists in water during 5 min, followed by gradient elution from 0 % to 60% acetonitrile in 4 min, then to 100 % acetonitrile in 11 min. Gradient elution with a rapidly increasing acetonitrile content, compatible with the ELSD, enables excellent separation. As on a C_{18}-bonded silica, the more methylated the compound is, the more its retention time increases. Contrary to the separation of β-CD, DM-β-CD and TM-β-CD obtained on C_{18}-bonded silica column with a polarimetric detector,[11] separation on Zorbax Phenyl column with the ELSD is obtained without baseline drift, with good efficiencies and in a short time (tr < 15 min). The separation enables a rapid qualitative determination of the TM-β-CD impurities.

Figure 3b shows the chromatographic fingerprint of a commercial TM-β-CD eluted in the same conditions as the standard mixture. The commercial TM-β-CD did not show any of the linear glucose impurities and contained very slight amounts of β-CD and DM-β-CD. Other chromatographic peaks, however, are present between the DM-β-CD peak and TM-β-CD peak. In view of the complexity of the β-CD alkylation method, these compounds should correspond to over-methylated homologues of DM-β-CD. To increase the resolution between DM-β-CD and TM-β-CD and thus to obtain a better distribution of partially methylated cyclodextrin chromatographic peaks, another chromatographic system has been studied.

Fig. 4a shows a very good isocratic separation of the standard mixture of β-CD, DM-β-CD and TM-β-CD on Zorbax Phenyl column with acetonitrile-water (45:55) as mobile phase. This isocratic analysis is very interesting because, until now, such a mixture could be analyzed only with a gradient elution on C_{18}-bonded silica column.[11] Under such elution conditions, β-CD was eluted close to the void volume and selectivity between DM-β-CD and TM-β-CD has significantly been improved in comparison with the gradient analysis. This system allows us to confirm that there is no β-CD and DM-β-CD in the commercial TM-β-CD but there are a high amount of compounds which have an intermediate methylation degree between those of DM-β-CD and TM-β-CD (Fig. 4b). By using the TM-β-CD calibration curve, the evaluation of the major impurity proportion was about 4.5 % (w/w). Considering all of the impurities, the degree of purity of TM-β-CD was about 94 %. So, commercially, it is more difficult to obtain a pure methylated cyclodextrin than its corresponding native cyclodextrin.

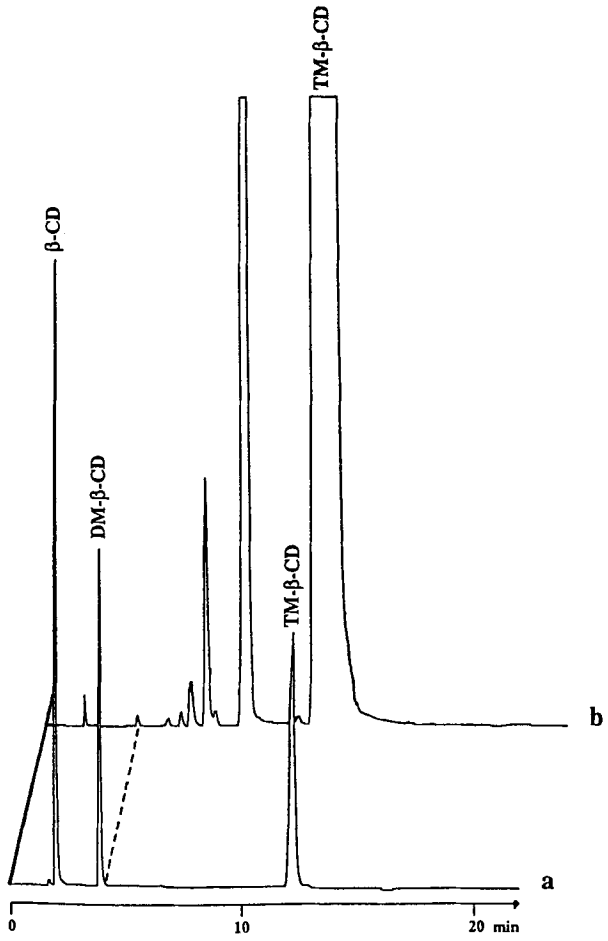

Figure 4. (a) Analysis of a standard mixture of β-CD, DM-β-CD and TM-β-CD. (b) Analysis of a commercial TM-β-CD (10 000 mg/L). Column, Zorbax Phenyl (250 x 4.6 mm I.D.); eluent, acetonitrile-water (45:55); flow-rate, 1 mL/min; evaporative light scattering detector.

CONCLUSION

This work presents the first application of the evaporative light scattering detector used to determine the purity of native (α-, β-, γ-CD) and derivatised (TM-β-CD) cyclodextrins. Although, strictly speaking, the response of ELSD is not directly proportional to the mass of the solute, the ELSD response factors

of these homologous compounds are highly similar, so it is possible to evaluate impurity levels, with an acceptable degree of approximation, without previous identification.

The use of ELSD permits one to obtain the separation of compounds with widely differing polarities, such as linear sugars, β-CD, and methylated β-CDs, as a result of its compatibility with gradient elution mode. Separation of cyclodextrins using Phenyl-bonded stationary phase and acetonitrile-water mobile phase offers selectivities that are different from those obtained using classical C_{18}-bonded silica column and allows one to achieve satisfactory isocratic and gradient analysis. The method provides good selectivity and sensitivity for the determination of cyclodextrins. Moreover, chromatographic systems presented here allowing the elution of impurities before the major product, could be used on the semi-preparative scale to purify TM-β-CD and can be used to compare different sources of β-CD and TM-β-CD and to measure batch to batch variation.

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