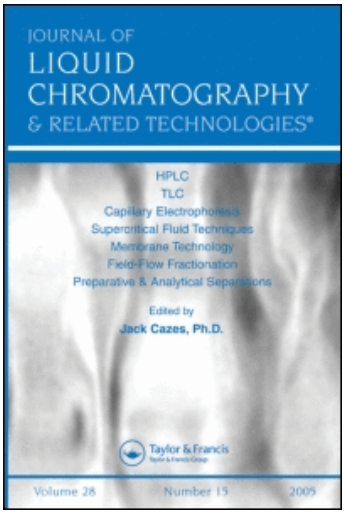

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ANALYSIS OF MALTODEXTRINS IN PLANT EXTRACTS BY LC USING EVAPORATIVE LIGHT SCATTERING DETECTION

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ABSTRACT

Two liquid chromatography methods using an evaporative light scattering detection (ELSD) have been developed to determine the carbohydrate profile of maltodextrins. The first method requires an octadecyl-bonded silica column and a methanol/water mobile phase. With the second method, an amino-bonded polymeric stationary phase and an acetonitrile/water eluent are used. In the two cases, a gradient elution was necessary to desorb the higher molecular weight polysaccharides. This elution mode was perfectly compatible with ELSD since no baseline drift was observed. These two methods were applied to quantify maltodextrins present in plant spray dried-powder of melilot and basil.

INTRODUCTION

Maltodextrins (MD) are starch hydrolysates consisting of α-D-glucose units linked by (1-4) glycosidic linkages (primarily) as well as by (1-6) linkages.[1,2] They have found a wide range of applications, particularly in the food industry and in cosmetics as, for example, an encapsulating agent to extend the shelf-life of easily oxidized compounds.[3,4] The physico-chemical properties of MD are dependent on the overall saccharide profile; the proportions of low molecular carbohydrates influence sweetness, viscosity, and fermentability.[5]

High performance liquid chromatography (HPLC) is the most commonly used method for the analysis of saccharides.[6-11] Carbohydrates being weakly acidic, a popular method consists of anion-exchange chromatography combined with pulsed amperometric detection with an electrode of noble metal.[12-14] However, this technique requires the use of high pH eluents causing epimerization and degradation of sugars. Another common system, well adapted to the separation of mono- and oligosaccharides, consists of an amino-bonded silica column and an acetonitrile/water eluent.[15,16] However, these supports have a short life time due to amino-bonded silica hydrolysis[17] and a reduction of the water content in eluent is necessary to stabilize these phases.[18]

Lack of chromophores in molecular structures of carbohydrates makes the coupling of HPLC with a UV detector very difficult. Refractive index detectors (RID) are frequently used, but they present several limitations since they are sensitive to temperature and pressure. Moreover, they are totally incompatible with gradient elution.[8] These disadvantages can be overcome using ELSD.[19] This detector is generally considered as a universal LC detector for analytes which are less volatile than the chromatographic eluent.[20-22]

Hernandez et al compared the two detection systems, RID and ELSD, for the determination of mono- and oligosaccharides in food vegetables using an amino-bonded silica column and an acetonitrile/water eluent.[23] RID was used with an isocratic elution mode and ELSD with a gradient elution mode. No statistically significant difference was observed between the two methods.

Herbreteau et al developed a method to separate oligosaccharides with an amino-bonded silica gel using a ternary gradient elution with ELSD.[18] The use of this detection system also enabled evaluation of phase hydrolysis.

In this work, we examine the possibility of separating and quantifying sugars present in MD by HPLC-ELSD using two packing materials: an octadecyl (C18) silica and an amino-bonded vinylic alcohol copolymer. This packing does not hydrolyse with the high percentage of water in the eluent, by contrast to an amino-bonded silica material. The other objective of this paper is to propose a simple method to quantify MD added in plant extracts as an encapsulating agent.

EXPERIMENTAL

HPLC Apparatus

The HPLC equipment consisted of a Merck-Hitachi LaChrom system equipped with a L7100 quaternary pump, a L7200 automatic injector and a D7000 interface (Merck, Darmstadt, Germany).

Piloting, data acquisition, and data processing were provided by HSM software.

The ELSD was a Sedere (Vitry-sur-Seine, France) model Sedex 75. The usual ELSD settings were as follows: drift tube 50°C, nebulizer gas pressure 3.5 bar.

The analytical columns were octadecyl-silica Spherisorb ODS-1 (250 x 4.6 mm i.d., particle size 5 μm) from Phase Separations (Norwalk, USA) and amino-bonded vinylic alcohol copolymer Astec (250 x 4.6 mm i.d.) from Advanced Separation Technologies (NJ, USA). The use of a precolumn containing the same packing material is recommended to extend the life of the analytical column, particularly when plant extract samples are injected.

Chemicals

HPLC-grade acetonitrile and methanol were from J. T. Baker (Noisy-Le-Sec, France). LC-grade water was prepared by purifying distillated water in an Elgastat UHQ II System from Elga (Villeurbanne, France). Glucose and maltose were obtained from Merck (Darmstadt, Germany). Maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose were from Sigma (Saint-Louis, MO, USA). Glucidex 6 and Glucidex 12 maltodextrins were supplied by Roquette (Lestrem, France).

The MD-6 and MD-12 maltodextrins were obtained from Cerestar (Neuilly-sur-Seine, France). Stock solutions of MD (1000 $mg.L^{-1}$) and oligosaccharides were prepared by weighing and dissolving them in LC-grade water. Solutions were stored at 4°C. Plant extracts (melilot and basil) were kindly supplied by Alban Muller (Montreuil, France).

Chromatographic Procedure

The analysis of MD using the C18 analytical column was performed with a methanol/water gradient at a flow-rate of 1 $mL.min^{-1}$. The gradient was 100% of water from 0 to 5 min, 30% at 25 min, 0% at 35 to 55 min.

With the NH_2 polymeric column, it was impossible to use a water/methanol eluent at flow-rates superior to 0.5 $mL.min^{-1}$ because the upper limit pressure was exceeded. Methanol was then replaced by acetonitrile. Different gradients were tested and the optimal one was as follows: 35% of water from 0 to 15 min, 50% at 25 to 35 min, 100% at 45 to 55 min. The flow-rate was 0.9 $mL.min^{-1}$.

Between two consecutive analyses, the stationary phase was equilibrated for twenty minutes with the initial mobile phase. Experiments were carried out at room temperature.

Sample Extraction

A quantity of plant extract equal to 50 mg was added to 10 mL of LC-grade water. This mixture was then stirred with a magnetic stick for five minutes. The liquid phase was then filtered through a syringe equipped with a filter for aqueous solutions of 45 μm porosity from Alltech (IL, USA). A 20 μL volume of this sample was then injected into the analytical column. The quantity of extracted MD was compared to a direct injection of the same theoretical quantity of MD in order to confirm a 100% extraction recovery.

RESULTS AND DISCUSSION

Determination of the Carbohydrate Profile of Maltodextrins

The analysis of MD was first investigated using a C18 analytical column with a methanol/water eluent. Chromatograms representing the fingerprint of the four MD are presented Figure 1. Peaks have been identified after comparison of chromatograms of standard saccharides injected in the same conditions as MD. Excellent resolution between oligosaccharides is observed. Sugars of degree of polymerisation (DP) 1 to 13 are resolved in less than 25 minutes. The pairs of peaks observed for hexaose and sugars of degree of DP 11 to 13 are attributed to α and β anomers.[5]

To elute the higher molecular weight carbohydrates, a 100% methanol mobile phase is required. In spite of the high variation in the composition of eluent (100 % of water to 0 % in 30 minutes), no baseline drift is observed, confirming compatibility of ELSD with gradient elution.

A second analytical method was developed using an amino-bonded polymeric column. In order not to overlap the upper limit pressure, methanol was replaced by acetonitrile. Several gradients of elution were tested to obtain adequate resolution of sugars from glucose to saccharides of DP 12-13. The gradient of the elution employed is described in the chromatographic procedure section.

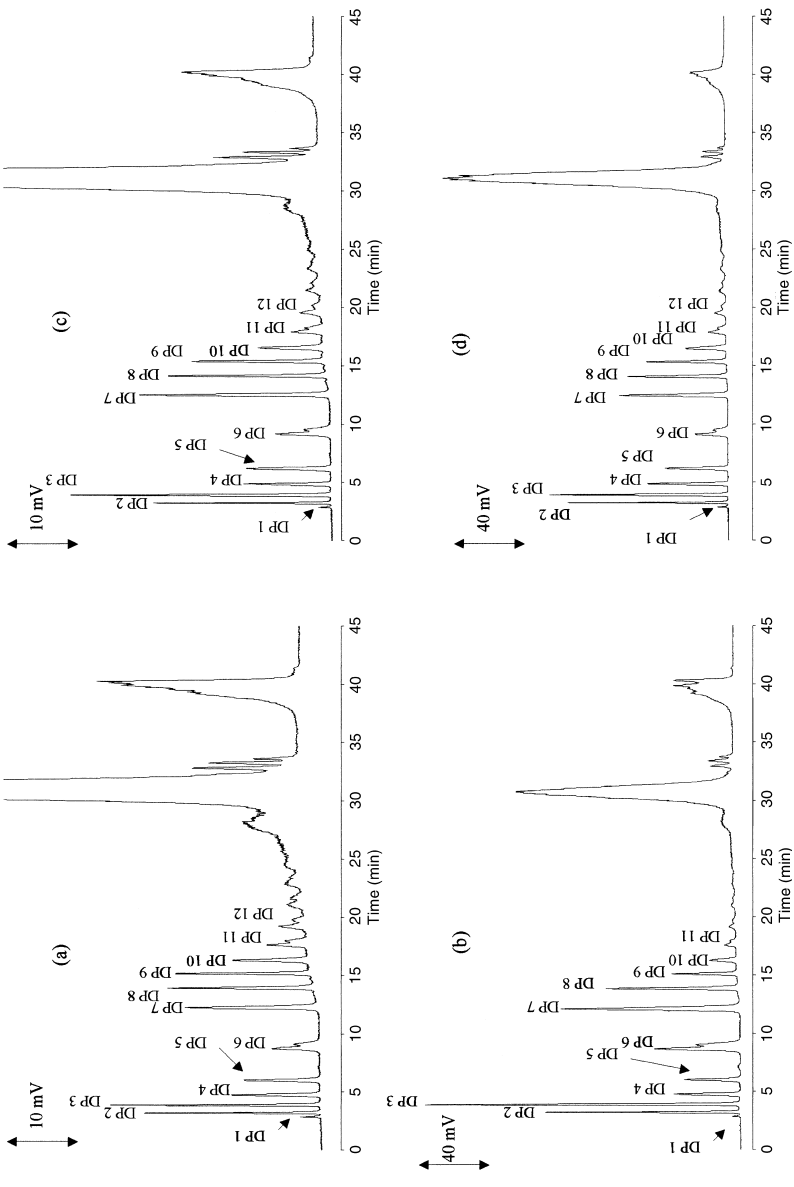

Figure 1. Chromatograms of maltodextrins at 1000 mg.L^{-1} (a) MD-6, (b) MD-12, (c) Glucidex 6, and (d) Glucidex 12, using an octadecyl-silica Spherisorb ODS-1 column. Mobile phase: methanol/water gradient, 100% of water from 0 to 5 min, 30% at 25 min, 0% at 35 to 55 min. Flow-rate: 1 mL.min^{-1}. Sedex 75 evaporative light scattering detector, drift tube temperature 50°C, photomultiplier 9, nebulizer gas pressure 3.5 bar. Numbers refer to degree of polymerization of the saccharides.

Figure 2 shows the chromatograms observed for the four MD. The retention mechanism being different from that observed with apolar silicas (normal-phase chromatography instead of reversed-phase chromatography), water is a better eluent than acetonitrile. To obtain excellent resolution between the first sugars, the initial mobile phase contains only 35% of water. At the end of the gradient, a 100% aqueous eluent is necessary to desorb the higher molecular weight saccharides.

A quantitative approach, consisting of evaluating the composition of each MD, was done by area normalization; all saccharides present in the MD being eluted from the column. This procedure supposes similar response factors for the different saccharides by ELSD, which was demonstrated in isocratic elution mode,[19] but not in gradient elution. In consequence, the calculated percentages by this area normalization method only allow an approximate composition of MD, and calibration from standard saccharides is recommended to minimize error percentage. The average weight percent compositions obtained from the two analytical methods are presented in Table 1. Injections of MD were done in triplicate on each analytical column.

The results clearly show a different carbohydrate profile from one MD to the other. The total weight percentage of saccharides from DP 1 to DP 12 is approximately 10% for MD-6 and Glucidex 6, whereas it is three times higher for Glucidex 12 and about four times higher for MD-12.

Plant Extracts

These two analytical methods were applied to quantify MD added to plant extract as encapsulating agent. After extraction of MD from the plant dried-powder (see sample extraction section for more details), a 20 μL sample was injected using the C18 analytical column or the amino-bonded polymeric one. Chromatograms obtained with the C18 apolar stationary phase, and corresponding to the injection of an extract of basil and of melilot, containing respectively, 10% or 20% (w:w) of MD-6 are presented in Figure 3.

Chromatograms corresponding to the injection of original plant extract samples are also presented (see chromatograms a and c Figure 3). For these two chromatograms, a noisy baseline is observed. Many molecules present in the plant material are extracted by water and eluted in the same time as the saccharides present in the MD. Consequently, chromatograms b and d corresponding to the plant extract containing MD are not useable.

Using the NH_2-bonded polymeric column, chromatograms are totally different (see Figure 4). For natural basil extract (chromatogram a), most of the molecules are eluted at the beginning of the chromatogram masking the peaks

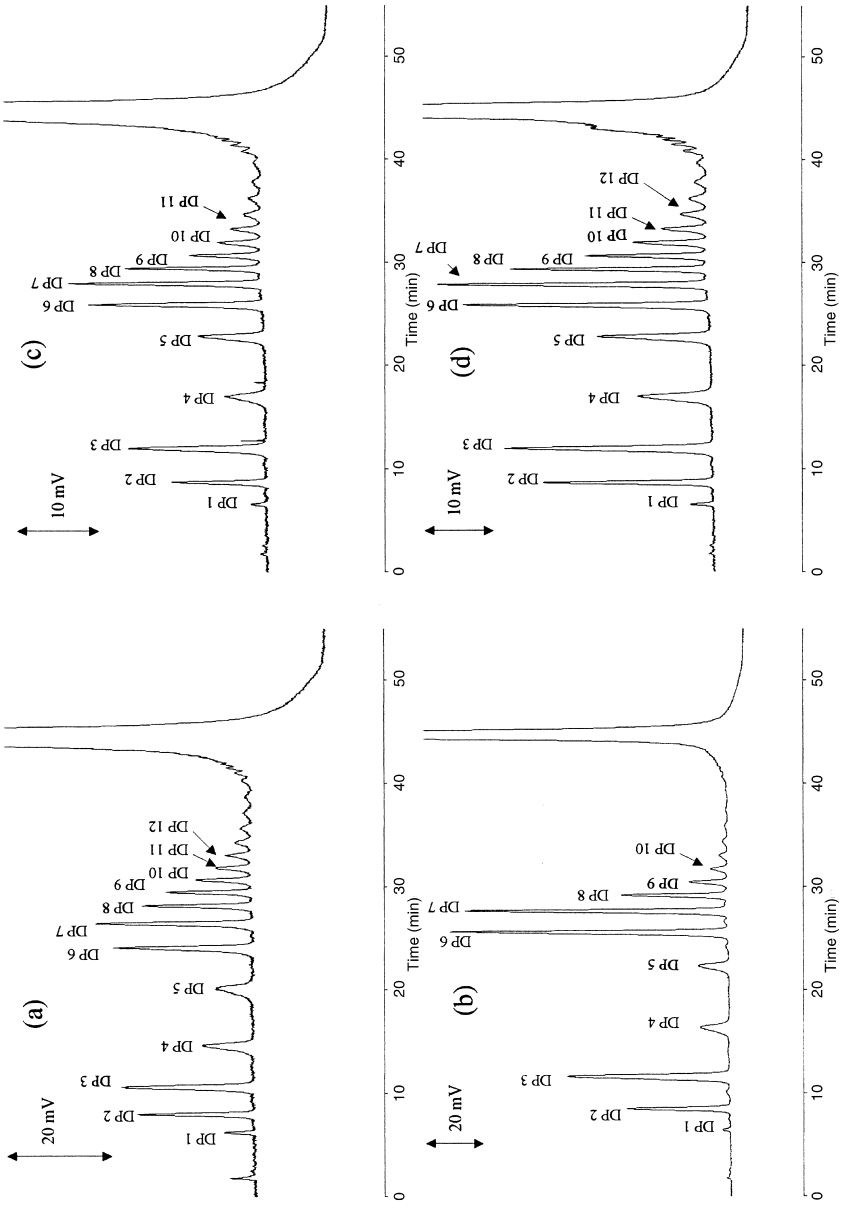

Figure 2. Chromatograms of maltodextrins at 1000 mg.L^{-1} (a) MD-6, (b) MD-12, (c) Glucidex 6, and (d) Glucidex 12, using an amino-bonded polymer Astec column. Mobile phase: acetonitrile/water gradient, 35% of water from 0 to 15 min, 50% at 25 min to 35 min, 100% at 45 to 55 min. Flow-rate: 0.9 mL.min^{-1}. Same detection conditions as Figure 1.

Table 1

Average Weight Percent Compositions of Maltodextrins Determined by Area Normalization. Mean Values from Three Replicate Experiments with the C18 Analytical Column and with the NH_2 Polymeric Phase

	Percentage (%) ± Standard Deviation			
	MD-6	**MD-12**	**Glucidex 6**	**Glucidex 12**
DP 1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
DP 2	0.9 ± 0.2	4.2 ± 0.7	0.9 ± 0.1	2.8 ± 0.3
DP 3	1.4 ± 0.3	9.4 ± 1.8	1.9 ± 0.2	4.7 ± 0.5
DP 4	0.9 ± 0.2	2.4 ± 0.2	0.8 ± 0.1	2.7 ± 0.3
DP 5	0.8 ± 0.1	2.1 ± 0.1	0.9 ± 0.1	2.4 ± 0.1
DP 6	1.1 ± 0.3	7.9 ± 1.6	1.2 ± 0.2	2.9 ± 0.5
DP 7	1.4 ± 0.1	9.3 ± 0.9	1.7 ± 0.1	4.0 ± 0.1
DP 8	1.1 ± 0.1	4.2 ± 0.1	1.2 ± 0.1	3.1 ± 0.1
DP 9	0.9 ± 0.1	1.9 ± 0.3	0.9 ± 0.2	2.4 ± 0.2
DP 10	0.7 ± 0.1	1.0 ± 0.2	0.6 ± 0.1	1.8 ± 0.1
DP 11	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	1.4 ± 0.2
DP 12	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	1.1 ± 0.2
Other Polysaccharides	89.8 ± 0.4	56.4 ± 4.7	88.9 ± 0.2	70.4 ± 1.7

of glucose to maltotetraose. No interferent is eluted between 20 and 35 minutes. Quantification of MD present in the basil extract is then possible by quantifying saccharides of DP from 5 to 10 whose retention times are comprised in this retention time interval (see chromatogram b). For melilot extract, similar results are observed (chromatograms c and d). The peaks of glucose to maltotetraose are hidden by impurities. Moreover, interferents are coeluted with maltoheptaose, maltononaose, and maltodecaose. Quantification of MD is then possible from three sugars (maltopentaose, maltohexaose, and maltooctaose).

The calibration curves for maltopentaose to maltodecaose have been determined, using the NH_2 column by reporting the logarithm of the surface area response versus the logarithm of the concentration of the MD. Although, the variation of the ELSD response is complex, a linear variation was demonstrated between surface area response and concentration obtained in double logarithmic coordinates."[24]

$$\text{Log } A = a.\log C + \log b,$$

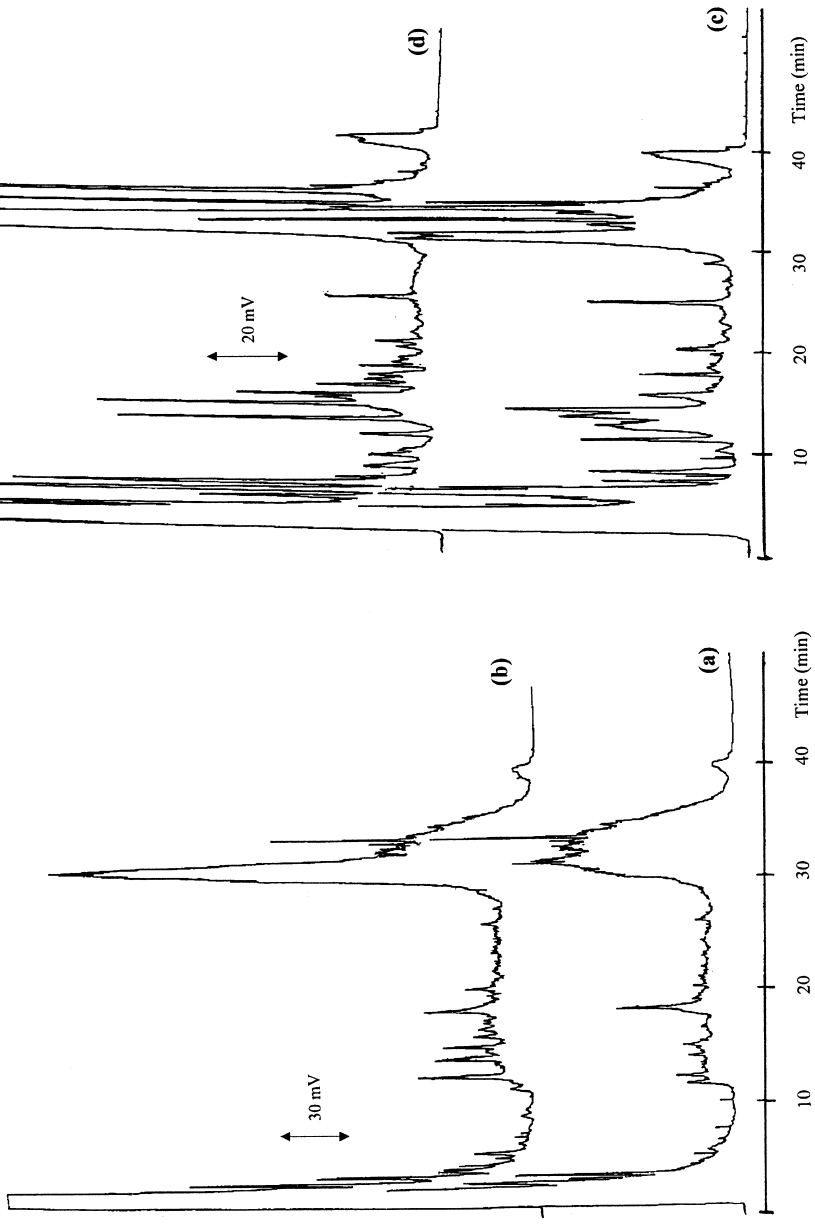

Figure 3. Injection of 20 µL of (a) a basil extract without maltodextrin, (b) a basil extract containing 10% (w:w) of maltodextrin MD-6, (c) a melilot extract without maltodextrin, and (d) a melilot extract containing 20% (w:w) of maltodextrin MD-6 using an octadecyl-silica Spherisorb ODS-1 column. Same analytical conditions as Figure 1.

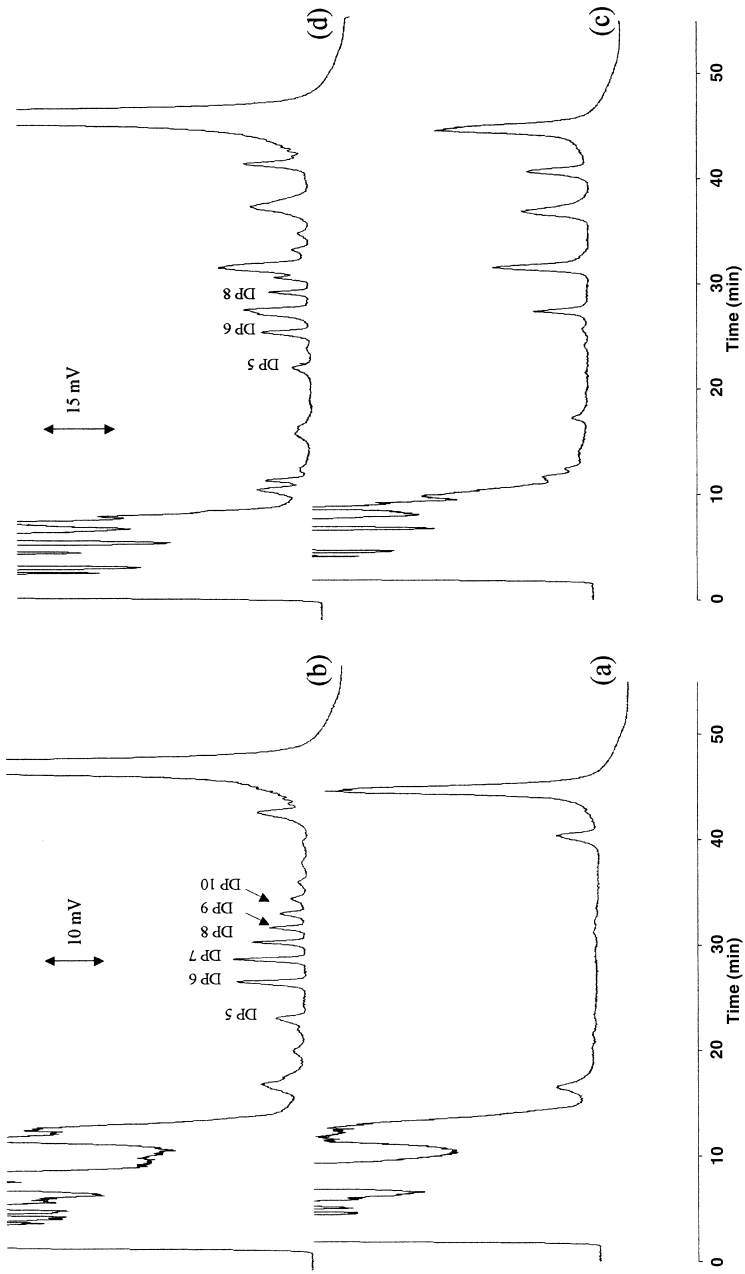

Figure 4. Injection of 20 µL of (a) a basil extract without maltodextrin, (b) a basil extract containing 20% (w:w) of maltodextrin MD-6, (c) a melilot extract without maltodextrin, and (d) a melilot extract containing 20% (w:w) of maltodextrin MD-6 using an amino-bonded polymer Astec column. Same analytical conditions as Figure 2.

Table 2

Parameters of Calibration Lines Obtained for Maltopentaose to Maltodecaose Using the Amino-bonded Polymeric Stationary Phase. Log A = a.log C + log b, A being the Surface Area Response of Saccharide and C the Maltodextrin Concentration

Sugars	Slope (a)	y-intercept (log b)	Correlation Coefficient
Maltopentaose	1.3410	1.2955	0.9990
Maltohexaose	1.5247	1.0270	0.9990
Maltoheptaose	1.5137	1.0445	0.9996
Maltooctaose	1.5291	0.7961	0.9992
Maltononaose	1.4672	0.8649	0.9990
Maltodecaose	1.5034	0.6065	0.9995

where A is the surface area response, C the solution concentration, a the slope of the response line, and b the response factor.

The concentration range was from 250 $mg.L^{-1}$ to 2000 $mg.L^{-1}$. This range was chosen to surround the concentration of MD equal to 1000 $mg.L^{-1}$ and corresponding to an initial percentage in the plant spray dried-powder of 20% (w:w). Injections were done in triplicate. The relative standard deviations (RSD) for surface area response were less than 3%. Quantitation parameters are reported in Table 2. Linear correlation coefficients are higher than 0.999. The procedure was applied to quantify MD-6 added to an extract of melilot or basil. Experimental concentrations of MD-6 were identical to theoretical values with an error percentage inferior to 3 %.

CONCLUSION

Determination of the carbohydrate profile of MD from glucose to saccharides of DP 12-13 was achieved using two simple HPLC procedures. The use of ELSD as detection mode permitted elution of all the saccharides present in MD in a single injection, as a result of its perfect compatibility with the gradient elution mode.

Quantification of MD present in plant spray dried-powder was possible with the amino-bonded polymeric material in spite of the initial presence of saccharides in the natural plant extract.

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REFERENCES

1. J. F. Kennedy, C. J. Knill, D. W. Taylor, in **Handbook of Starch Hydrolysis Products and their Derivatives**, M. W. Kearsley, S. Z. Dziedzic, eds., Blackie, London, 1995, Ch.3, p. 65.

2. I. S. Chronakis, Crit. Rev. Food Sci., **38(7)**, 599-637 (1998).

3. C. S. Setser, W. L. Racette, Crit. Rev. Food Sci. Nutr., **32(3)**, ,275-297 (1992).

4. J. R. Alexander, **Maltodextrins -Production, Properties and Applications, in Starch Hydrolysis Products**, E. W. Schenck, R. E. Hebeda, eds., VCH, New York, 1992, p. 233.

5. N. W. H. Cheetham, P. Sirimanne, W. R. Day, J. Chromatogr., **207**, 439-444 (1981).

6. A. Meunier, M. Caude, R. Rosset, Analusis, **14**, 363-377 (1986).

7. M. Verzele, F. Van Damme, J. Chromatogr., **362**, 23-31 (1986).

8. K. B. Hicks, Adv. Carbohydr. Chem. Biochem., **46**, 17-72 (1988).

9. P. E. Shaw, **Handbook of Sugar Separation in Food by HPLC**, CRC Press, Boca Raton, Florida, 1988.

10. S. C. Churms, J. Chromatogr., **500**, 555-583 (1990).

11. B. Herbreteau, Analusis, **20**, 355- 374 (1992).

12. R. D. Rocklin, C. A. Pohl, J. Liq. Chromatogr., **6**, 1577-1590 (1983).

13. M. R.Hardy, R. R. Townsend, Y. C. Lee, Anal. Biochem., **170**, 54-62 (1988).

14. T. J. Lu, W. R. Lacourse, L. Jane, Starch/Stärke, **49**, 505-511 (1997).

15. C. Martin-Villa, C. Vidal-Valverde, E. Rojas-Hidalgo, J. Food Sci., **47**, 2086-2088 (1982).

16. T. Van Den, C. J. Biermann, J. A. Marlett, J. Agric. Food Chem., **34**, 421-425 (1986).

17. D. Karlesky, D. C. Shelly, I. Warner, Anal. Chem., **53**, 2146-2147 (1981).

18. B. Herbreteau, V. Villette, M. Lafosse, M. Dreux, Fresenius J. Anal. Chem., **351**, 246-250 (1995).

19. M. Dreux, M. Lafosse, "Evaporative Light Scattering Detection of Carbohydrates in HPLC", in **Carbohydrate Analysis : High Performance Liquid Chromatography and Capillary Electrophoresis**, J. Chrom. Library, Vol. 58, Z. El Rassi, ed., Elsevier, Amsterdam, 1995, pp. 515-540.

20. M. Dreux, M. Lafosse, Spectra 2000, **suppl. 151**, 16-21 (1990).

21. M. Dreux, M. Lafosse, Spectra 2000, **153**, 24-32 (1990).

22. M. Dreux, M. Lafosse, Analusis, **20**, 587-595 (1992).

23. J. Lopez Hernandez, M. J. Gonzalez-Castro, I. Naya Alba, C. de la Cruz Garcia, J. Chromatrogr. Science, **36**, 293-298 (1998).

24. A. Stolyhwo, H. Colin, M. Martin, G. Guiochon, J. Chromatogr., **288**, 253-275 (1984).

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