Separation and quantification of carbohydrates by high performance thin layer chromatography – HPTLC

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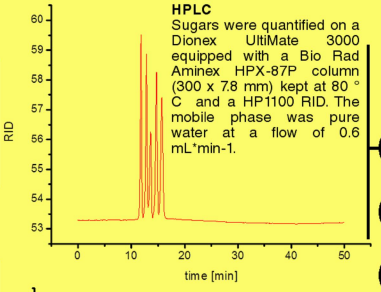
INTRODUCTION

The need for fast and accurate analysis methods of plant-derived low-molecular weight carbohydrates is associated with the fact that the development of biorefineries is rapidly progressing:
Since plant material can differ strongly in its composition, fast and robust screening methods are of great importance. A disturbing matrix, consisting of dissolved lignin, furfural, formic and acetic acid, sugar acids, various oils and other products can falsify results or even can clog columns or capillaries which lead to increased maintenance needs and costs. Quantification of the sugar components after hydrolysis is nowadays mainly done by chromatographic methods. However, these well established techniques are still time consuming and expensive when it comes to analysis of higher sample numbers.
HPTLC is an enhanced TLC method which includes fully automated steps using high performance silica layers for increased sensitivity.

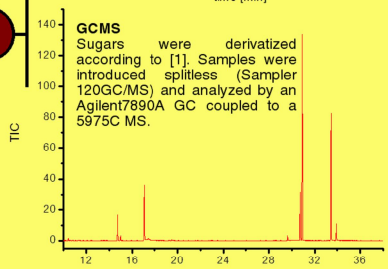
HPLC

Sugars were quantified on a Dionex UltiMate 3000 equipped with a Bio Rad Aminex HPX-87P column (300 x 7.8 mm) kept at 80 °C and a HP1100 RID. The mobile phase was pure water at a flow of 0.6 mL*min-1.



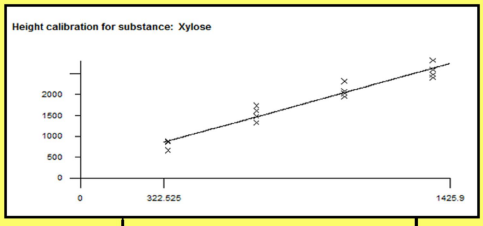
GCMS

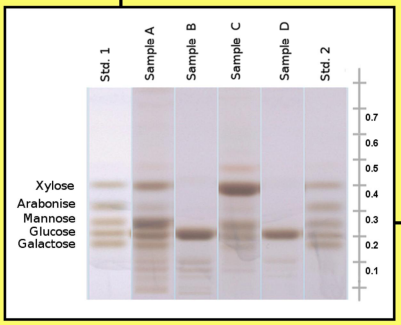
Sugars were derivatized according to [1]. Samples were introduced splitless (Sampler 120GC/MS) and analyzed by an Agilent7890A GC coupled to a 5975C MS.

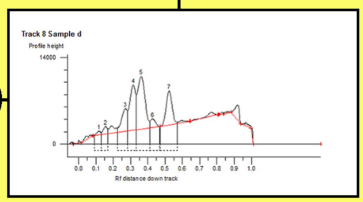


HPTLC

Planar chromatography was performed on impregnated HPTLC plates silica gel 60. Samples and standards were applied by the Automatic TLC Sampler 4 (ATS 4, Camag). Chromatography was carried out in an Automated Developing Chamber acetonitrile: 1-pentanol: water 4:1:1 (v/v/v). For post-chromatographic derivatization anilin diphenylamin dye was applied. Plate images were documented using the Camag TLC visualizer. Quantitative evaluation of the image was done by Camag VideoScan 1.02.00 via peak height.







RESULTS

HPTLC offers a lot of benefits when compared to other methods: low running costs, high sensitivity, high throughput or the ability to simultaneously isolate and identify the analytes, to name but a few. Quantification of plant-derived carbohydrates needs methods which are reliable, especially in routine analysis, as e.g. for control of fermentable sugars. Methods which are simple, rapid and capable of being run in large numbers are of particular interest. Paper chromatography (PC) and TLC have been extensively used in the analysis for sugars [2]. However, quantification of sugars is nowadays mainly done by GC or LC approaches. In a first approach we showed that results obtained by HPTLC are comparable to those from HPLC or GCMS methods.

CONCLUSION

We demonstrated the usefulness of planar chromatography in routine quantification of sugars derived from plant hydrolyzates. Especially in industry (e.g. for control of fermentation processes) the accurate and fast quantification of the sugar content is of great importance. As matrix problems are a minor issue in HPTLC, the method is very flexible with regard to sample origins and impurities. Within two hours, 16 samples can be fully analyzed, which is by far superior to HPLC or GCMS. The comparison with classical methods for quantification of wood sugars showed HPTLC to stand fully emancipated among alternative separation techniques. A full validation of the HPTLC

	Sample A			Sample B			Sample C		
	HPTLC [g*l-1]	HPLC [g*l-1]	GCMS [g*l-1]	HPTLC [g*l-1]	HPLC [g*l-1]	GCMS [g*l-1]	HPTLC [g*l-1]	HPLC [g*l-1]	GCMS [g*l-1]
Arabinose	13.9	12.8	-	10.12	28.5	7.6	-	-	-
Galactose	23.9	22.0	28.3	28.3	-	8	-	-	-
Glucose	29.6	46.2	47.1	20.8	32.5	11.9	38.9	115.9	112.3
Mannnose	44.4	95.1	93.4	27.8	49.3	-	1.2	-	-
Xylose	34.6	54.1	57.3	60.4	231.6	248.6	2.1	0.4	-

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