A Densitometric Method for the Determination of Three Clinically Important Monosaccharides in Urine

Pascale Poukens-Renwart and **Luc Angenot***

Key Words:

Densitometry
HPTLC
Monosaccharides
Urine

Summary

A quantitative, densitometric, thin layer chromatographic method has been developed for the determination of three clinically important monosaccharides in urine, after desalting. Xylose, 3-*O*-methylglucose, and rhamnose were estimated quantitatively in urine; arabinose was used as internal standard. The densitometry was performed at λ = 400 nm after a visualizing reaction on HPTLC plates.

In all cases, coefficients of variation of the method were less than 2.7 %.

1 Introduction

In studying the changes induced by antiinflammatories in human small intestine permeability, we have measured, in a bulk 6 h urine sample, the remaining fraction of an ingested dose of the three monosaccharides: xylose, 3-*O*-methylglucose and rhamnose; arabinose was added as internal standard. The technique described in this paper is based on that published by *Menzies* [1] and incorporates a number of improvements, the most important of which are:

- the use of HPTLC plates instead of TLC plates, thus improving the resolution and enabling a better separation, especially between the 3-*O*-methylglucose and rhamnose [2, 3]
- modification of the visualizing reagent and coupling with an automatic dipping procedure (time of immersion, 2 seconds) [4, 5]
- quantitative determination using a CD 60 TLC Scanner (Desaga) to scan the range λ = 200 to 700 nm. The mean maximum wavelength for the four monosaccharides is λ 400 nm.

2 Experimental

2.1 Chemicals

The monosaccharides used in this investigation were purchased from Janssen Chimica.

2.2 Instrumentation

A CD 60 TLC Scanner (Desaga), a 20 x 10 cm dipping chamber (Camag), an HP Thermoplate (Desaga), and graduated disposable micropipettes (1-2-3-4-5 μl ± 0.1 μl at 20 °C, IntraMark) were used in the study. Chromatography was performed on HPTLC Silicagel 60 (20 x 10 cm) precoated glass plates (Merck).

2.3 Preparation of Solutions

2.3.1 Preparation of Standards

Preparation of Internal Standard Solution

500 mg Arabinose and 750 mg Thiomersal were dissolved in 50.0 ml water

Preparation of Stock Solution of Monosaccharides

500 mg of each monosaccharide (xylose, 3-*O*-methylglucose and rhamnose) and 375 mg Thiomersal were dissolved in 25.0 ml water. The preparation of standard solutions (ST) is detailed in Table 1. The concentrations of the standards were established after trials enabling us to determine up to which concentration the relationship between detector response

P. Poukens-Renwart and L. Angenot, Department of Pharmacognosy, Institute of Pharmacy, University of Liège, rue Fusch 5, B-4000 Liège, Belgium

© 1991 Dr. Alfred Huethig Publishers

and sample concentration remained linear: it was observed that above 1 μg/4 μl the relationship was no longer linear.

2.3.2 Preparation of Samples

To standardize the method, the monosaccharides were added to urine samples at the same concentrations as those expected in the urines to be analyzed. The assays were performed in the presence of glucose, fructose and galactose to show that there was no interference between these three monosaccharides and those being assayed. For the assays, each urine sample collected to be analyzed was made up to a standard volume of one liter; in this way, the monosaccharide concentrations could be read directly from the calibration graph. 0.5 ml of the internal standard solution is introduced into a 50.0 ml volumetric flask and made up the mark with the urine to be analyzed.

2.3.3 Desalting

This operation is performed on the different standard and sample solutions. 5 ml of each solution is added to 1.6 g of an ion exchange resin (Duolite MB 6113 from B.D.H.) converted

Table 1

Preparation of standard solutions

	ST_1	ST_2	ST_3	ST_4
Stock solution of monosaccharides	1 ml	0.5 ml	0.75 ml	0.5 ml
Stock solution of arabinose	2.5 ml	1 ml	1 ml	0.5 ml
Total volume	250 ml	100 ml	100 ml	50 ml
Concentration in 4 μl	0.32 μg	0.4 μg	0.6 μg	0.8μg

to its acetate form [1]. The mixture is shaken for 3 min and then centrifuged for 3 min (2000 r.p.m.). The supernatant solutions can be used for spotting on to the plates.

2.4 Chromatographic Procedure

4 μl of each supernatant solution were spotted (circular deposits) 15 mm from the lower edge of the chromatoplate. Three consecutive runs using ethyl acetate-pyridine-acetic acid-water (75 + 15 + 10 + 10; *v/v*) [6] were required to separate the four monosaccharides. The migration distance was 8 cm; each run took 50 min.

2.5 Detection

Each plate was left overnight to ensure complete evaporation of the mobile phase and then immersed for 2 s in aminobenzoic acid reagent (dissolve 1 g 4-aminobenzoic acid in 18 ml glacial acetic acid and add 20 ml water and 1 ml 85 % phosphoric acid; immediately before use, dilute with acetone in the ratio 2:3 [4]).

Table 2

Accuracy data for different monosaccharide levels in urine, after visualizing reaction with the reagent used by *Menzies* [1], and without automatic dipping procedure

	Concentration, *u* [μg/4μl][a)]	Amount found [μg] $x \pm S.D.$ $n = 20$	Accuracy $(x\text{-}u)/u \times 100 \pm S.D.$
Xyl	0.4	0.4100 ± 0.0434	2.5030 ± 2.6931
	0.8	0.8121 ± 0.0294	1.5120 ± 3.2140
Meglc	0.4	0.4328 ± 0.0259	8.2107 ± 3.6241
	0.8	0.8831 ± 0.0561	10.3908 ± 3.7001
Rha	0.4	0.4364 ± 0.0285	9.1021 ± 2.934
	0.8	0.9072 ± 0.1213	13.4102 ± 3.041

a) Xyl = xylose; Meglc = 3-*O*-methylglucose; Rha = Rhamnose

Thanks to the use of the automatic dipping chamber, there was no tailing of the zones. After drying the plate at room temperature for 10 min, the colors were produced by heating at 120 °C for 20 min. Arabinose and xylose appear as red-brown spots and 3-*O*-methylglucose and rhamnose as brown spots.

The monosaccharides were quantitated by densitometric scanning of the chromatogram in the reflectance mode (slit width: 0.04 x 6 mm, λ = 400 nm).

3 Results and Discussion

The dependence of densitometer response on absorbance of the four monosaccharide standard solutions was checked. The concentration of the standards was established after different trials enabling us to determine up to which concentration the relationship between absorbance and concentration remained linear. Densitometer scans were plotted as peak area against absorbance at λ = 400 nm; it was observed that above 1 μg/4 μl, the relationship was no longer linear, making it absolutely necessary to dilute the urine samples collected before analysis. The use of the new visualizing reagent gave red-brown or brown spots on a colorless instead

Table 3

Accuracy data for different monosaccharide levels in urine after visualizing reaction with the aminobenzoic acid-acetone reagent [4]

	Concentration, *u* [μg/4μl][a)]	Amount found [μg] $x \pm S.D.$ $n = 20$	Accuracy $(x\text{-}u)/u \times 100 \pm S.D.$
Xyl	0.4	0.4002 ± 0.0108	0.0488 ± 2.6954
	0.8	0.7993 ± 0.0178	−0.0213 ± 2.2255
Meglc	0.4	0.3946 ± 0.0096	−1.2838 ± 2.3914
	0.8	0.7959 ± 0.0222	−0.5094 ± 2.7710
Rha	0.4	0.4002 ± 0.0110	0.0763 ± 2.7760
	0.8	0.8069 ± 0.0203	0.8631 ± 0.9290

a) xyl = xylose; Meglc = 3-*O*-methylglucose; Rha = Rhamnose.

of a yellow background. Because of this, there was no interference during the measurement and the results were better than those obtained previously **(Tables 2 and 3).**

The wavelength measurement is the mean maximum wavelength obtained after recording absorption spectra of the four monosaccharides at different wavelength.

In order to determine the accuracy of the method, two sample solutions were prepared containing, respectively, 0.4 and 0.8 μg/4 μl of each monosaccharide, with the appropriate concentration of internal standard. The mean results of twenty replicate analyses performed for each concentration are reported in Table 3.

In all cases coefficients of variation of the method, obtained with the new reagent, were lower than 2.7 %.

4 Conclusion

Xylose, 3-*O*-methylglucose and rhamnose can be quantitatively estimated in urine, in the presence of glucose, galactose and fructose, by direct densitometry. This method is precise and accurate.

Acknowledgments

This work was supported by the FNRS (National Fund for Scientific Research)

References

[1] *I.S. Menzies, J. N. Mount,* and *M. J. Wheeler,* Ann. Clin. Biochem. **15** (1978) 65 – 76.

[2] *A. K. Singh, K. Granley, M. Ashraf,* and *U. Mishra,* J. Planar Chromatogr. **2** (1989) 410 – 419.

[3] *J. Grösz* and *W. Braunsteiner,* J. Planar Chromatogr. **2** (1989) 420 – 423.

[4] *H. Jork, W. Funk, W. Fischer,* and *H. Winner.* Thin Layer Chromatography, 1A, VCH, Weinheim (1990) 154 – 155.

[5] *H. Jork, W. Funk, W. Fischer,* and *H. Winner.* Thin Layer Chromatography, 1A, VCH, Weinheim (1990) 82 – 86.

[6] *I. Bjarnason, P. Smethurst, C. G. Fenn, C. E. Lee, I. S. Menzies,* and *A. J. Levy.* Digestive Diseases and Sciences, **34** (1989) 407 – 411.

Ms received: December 29, 1990
Accepted by SN: January 25, 1991