RAPID DETERMINATION OF LACTULOSE IN DAIRY PRODUCTS USING ULTRAPERFORMANCE CONVERGENCE CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY

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INTRODUCTION

Lactose is the main carbohydrate occurring in milk，but different processing technology, for example, high temperature sterilization, will lead to the conversion of lactose into lactulose. Lactulose represents the most widely studied index for distinction of heat-treated milk and for evaluating the heat load to which milk was subjected.

In addition, sucrose will be added into processed foods, such as yogurt and milk beverage to improve the flavor of the products by dairy companies. Several methods have been reported and applied to detect lactulose in milk, including enzymatic [1], GC and HPLC [2] methods. The major disadvantages of these methods are laborious, time consuming and less sensitive. One of the main problems in measuring lactulose in dairy products is the coexistence of lactose, sucrose and lactulose, which have a similar retention time using HPLC. In particular, the amount of sucrose and lactose are more than two orders of magnitude larger than that of lactulose.

Here we present a rapid and sensitive quantification of lactulose in dairy products based on ultra-performance convergence chromatography (UPC^2) coupled with an ACQUITY QDa. The target analytes were separated well on a Waters ACQUITY UPC^2 Torus DEA column based on MS chromatography using selected ion recording mode. Calibration curve of lactulose was quadratic ($r^2 \geq 0.99$) within the range of 0.1 to 10.0 mg L^{-1}. The relative standard deviation (RSD, n=6, 10.0 mg L^{-1}) of retention time is 0.2 % and that of peak area is 0.5 %. The limits of detection and quantification of the lactulose were 0.75 and 2.5 mg L^{-1}, respectively.

METHODS

Standard Preparation

A 10 mg mL^{-1} stock of the standard lactulose was prepared in water. The stock solution was used to prepare individual matrix standard working solutions at levels 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 mg L^{-1}.

Dairy product samples

Different types of commercial dairy products were analyzed with the proposed methodology including milk beverage, yoghourt, pasteurized milk and reconstituted milk. The dairy products for test were purchased randomly from a local grocery store. Samples were refrigerated at 4°C and opened just before analysis.

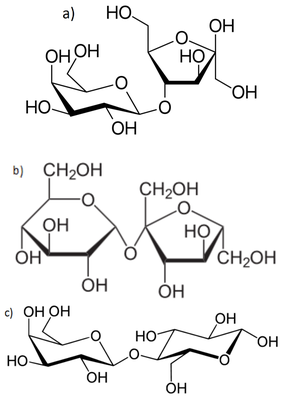

Figure 1. *Chemical structures of lactulose (a), sucrose (b) and lactose (c)*

Sample preparation

Samples were prepared in the following way: 0.2 gram of homogenous and representative sample was weighted into 15 mL centrifuge tube. Then, 0.8 mL water and 4 mL methanol/ acetonitrile (1/3, v/v) were added to each tube; the mixture was vortexed for 30 s. After that, the extract was centrifuged at 14 000 rpm for 10 min at 4°C. After centrifugation the supernatant is injected into the UPC^2 system.

UPC^2 conditions

Instrument: ACQUITY UPC^2 System
Run time: 10.0 min
Column: ACQUITY UPC^2 Torus DEA Column (130Å, 1.7 µm, 3 mm x 100 mm)
Column Temp: 40°C
Injection vol.: 1 µL
Mobile phase: A) supercritical CO_2
B) methanol/water (97/3, v/v) with 0.1% ammonia
Flow rate: 1.0 mL min^{-1}
Co-solvent: methanol with 0.4% formic acid
Co-solvent flow rate: 0.2 mL min^{-1}
ABPR: 2 000 psi

Table 1. *Gradient elution conditions used for quantification of analytes in samples.*

Time (min)	%A	%B	Curve
Initial	78	22	Initial
4.00	74	26	6
6.30	73	27	6
6.70	50	50	6
7.50	50	50	6
8.00	78	22	6
10.00	78	22	6

MS conditions

MS system: ACQUITY QDa
Ionization mode: ESI+
Capillary voltage: 0.8 kV
Cone Voltage: 20 V
Probe temp: 600°C
Acquisition Rate: 5 Hz
Scan Type: SIR
m/z $[M+Na]^+$: 365

Informatics

UPC^2-MS data was analyzed by Empower 3 FR3 .

ACQUITY UPC^2 System, ACQUITY UPC^2 Torus DEA Column, and QDa Mass Detector

RESULTS

Method Performance

Confirmation

A small and robust mass detector with high sensitivity compared with detector refractive index and evaporative light scattering detector was selected in this study. Quantification of lactulose was performed by employing selected ion recording (SIR) mode (M+Na: m/z 365). Lactulose, sucrose and lactose were well resolved from the peaks with resolution values (Rs) more than 1.5 (a baseline resolution has been achieved, Figure. 2). It is worthing noting that lactulose peak (RT=6.151 min) has a shorter retention time (RT) than sucrose peak (RT=6.961 min) and lactose peak (RT=7.344 min), and the tail effect is lowered.

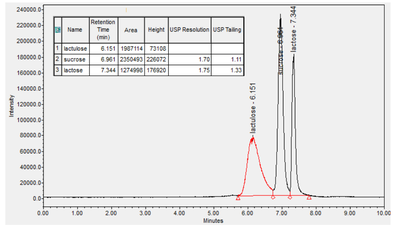

Figure 2. *UPC^2 separation of lactulose, sucrose and lactose in standard solution (5 mg L^{-1})*

Calibration Curves

Since lactulose is the typical lactose-derived isomeric disaccharide under severe heating but absent in raw milk, raw milk was chosen as a blank to prepare the matrix solution. Under the optimal conditions, matrix standard working solutions at six concentrations (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 mg L^{-1}) were analyzed. A linear relationship between the concentrations of lactulose and peak areas was obtained in the range of 0.1 to 10.0 mg L^{-1} (r^2=0.99).

LOD, LOQ and Recovery

Limit of detection (LOD) and limit of quantification (LOQ) was determined by the chromatographic noise obtained for a blank of matrix solution.

Results show that the LOD and LOQ were 0.03 and 0.1 mg L^{-1} in matrix solution, respectively. Considering the dilution factor of sample pretreatment, the LOD and LOQ could be lower than 0.75 and 2.5 mg L^{-1} in milk and yoghourt samples. The analyte recovery of this procedure was evaluated by fortified blank samples at 100 mg kg^{-1} with three replicates. Recovery was determined by comparing the average peak areas of spiked samples to those of matrix-matched standard solutions. The average recovery was 93.1 % and relative standard deviations was less than 2.1% for pasteurized milk, indicating good recovery. Compared with the methods developed using HPLC, the proposed developed using HPLC, the proposed method shows some superiorities, such as shorter retention time, less solvent consumption, better peak resolution and higher sensitivity.

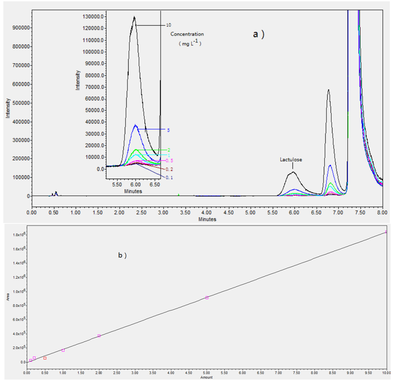

Figure 3 *a) Chromatographic peak of lactulose in matrix solution at different concentrations in the range of 0.1 to 10.0 mg L^{-1}; b) Standard curve between the peak area and lactulose concentrations in the range of 0.1 to 10.0 mg L^{-1}*

Lactulose Content in Samples

For food control authorities, lactulose and β-Lg concentrations are useful tools to distinguish between UHT and in-container sterilised milk [3-5]. Thus, the IDF and the EU suggest 600 mg L^{-1} lactulose as an upper limit for UHT milk associated with a β-Lg concentration of 50 mg L^{-1}. As shown in Figure 4., different types of commercial products were analyzed with the proposed methodology including milk beverage, yoghourt, pasteurized milk and reconstituted milk. There was only one negative result in nine samples. But all the concentration of lactulose in positive samples were lower than 600 mg L^{-1}.

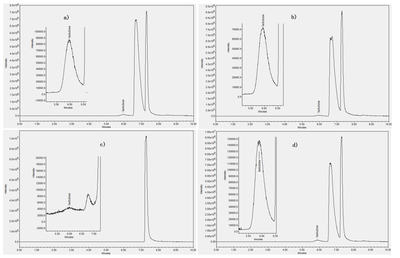

Figure 4. *UPC^2 spectrum of lactulose in milk beverage (a), yoghourt (b), pasteurized milk (c) and reconstituted milk (d)*

CONCLUSION

- **The UPC^2-MS assay provided rapid, sensitive quantification of lactulose in milk and yoghourt, which could be applied to differentiating heat treated milks.**
- **Almost all examined dairy product samples were positive in lactulose test, but did not exceed the limit of quantitation.**

References

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