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P.60 LIQUID CHROMATOGRAPHY ASSAY FOR GLIADINS QUANTIFICATION: APPLICATION TO GLUTEN-CONTAINING AND "GLUTEN-FREE" FOOD PRODUCTS

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In the present work a high performance liquid-chromatography UV/Vis method was developed to quantify gliadins in foods. Gliadins are prolamins and are one of the constituents of wheat gluten (approximately 50%). These compounds have particular importance in food analysis since they are responsible for the celiac disease, which is an intolerance or hypersensitivity to ingested prolamins. The only known treatment for celiac disease is a gluten free diet. Therefore, the development of analytical methods to assess the gliadins contents in food products is crucial, namely in those labeled as "gluten-free". A "gluten-free" alimentary product cannot contain more than 100ppm of gluten, which corresponds to 50ppm of gliadins.
Fifteen wheat based foods (7 "gluten-free" and 8 gluten-containing food samples, according to the label) were purchased in commercial supermarkets and analyzed using the HPLC method developed. The samples studied included flours, biscuits, breakfast cereals and bread.
The HPLC equipment consisted of a Varian chromatographic system, equipped with a Varian Prostar 220 pump and a 7725i Rheodyne manual injector with a 10μL loop. A Varian ProStar 330 Photodiode Array detector was used. The equipment was controlled using the Star Chromatography Workstation software (version 4.5), which also controlled the solvent gradient, the data acquisition and data processing. The chromatographic separation was performed with a chromatographic column PLRP-S (polystyrene divinylbenzene stationary phase, particle size 8 microns, pore 300Å and 150×4.6mm id). The column was placed inside an oven (Jones, Model 7981) and kept at 40±0.1°C. The best chromatographic resolution for the gliadins was achieved using a gradient elution with acetonitrile/TFA/water. The elution was performed at a constant flow of 0.6mL/min and at a temperature of 40±0.1°C. The detection was made at a wave-lenght of 210nm. Each sample analysis took 30min.
The gliadins concentrations of the calibration standard solutions (different standard gliadin masses dissolved in ethanol/water mixed in the proportion of volume 7/3) were confirmed using a Pierce Coomassie Plus kit test.
The gliadins were extracted from the food samples using an ethanol/water solution (70:30, v/v) solution and, after vortex homogenization and centrifugation, the solutions were filtered through a Whatman 0.2μm nylon filter, before HPLC analysis.
Calibration results showed a linear relationship between peak areas related to gliadins and concentrations with a correlation coefficient of 0.9993. The calibration curve was established by the external standard calibration method. The dynamic range of gliadins concentrations studied was between 50 and 1309ppm. The detection and quantification limits obtained were 46.5 and 140.91ppm of gliadins, respectively. Data refer to the overall area of all peaks. The values obtained for repeatability and intermediate precision were acceptable since the relative standard deviation percentage values were lower than 4%.
The gliadins peaks in the food samples were identified based on the retention times obtained for the standard solutions of gliadins and by analysis of the UV spectrum associated to each peak. Results confirmed the presence of gliadins in all food samples indicating gluten-containing label. Moreover, gliadins were also detected in two "gluten-free" food samples. Furthermore, in one of

them it was also possible to quantify the gliadin level, which was greater than 50ppm, indicating that the "gluten-free" label was incorrect.
The results obtained showed that the HPLC method developed in this work can be a useful tool for assessing gliadin contents in "gluten-free" products, although some additional effort must be made to decrease the detection and quantification limits of the proposed method.

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Liquid chromatography assay for gliadins quantification:
Application to gluten-containing and "gluten-free" food products

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OBJECTIVES

HPLC analytical method implementation for Gliadin analysis in foodstuffs, especially those declared as "gluten-free"

INTRODUCTION

- Celiac disease (CLD) is an autoimmune-mediated disorder, which is triggered by the ingestion of prolamins, namely gliadins
- The gluten wheat proteins contain about 50% of gliadins
- CLD prevalence is in the range of 0.5 to 1.0% in Europe and U.S.A.
- CLD may revert if a strict "gluten-free" lifelong diet is established
- "Gluten-free" and "low gluten content" products must not contain more than 20 and 100 ppm of gluten, respectively
- "Gluten-free" foods must be analytically controlled

SAMPLES

15 Food samples

Food	Label indicating Food Gluten Containing	Label indicating Food Gluten Free
Flour	2	1
Bread	1	1
Baby food formula	2	2
Wafer	2	2
Breakfast cereals	1	1

GLIADIN SOLUTION STANDARDIZATION

Coomassie Plus – The Better Bradford™ Assay Kit

BioTek Instrument EL×800 Microplates

Software Gene5

Detection at 595nm

Albumine standard solutions

HPLC SYSTEM

Varian Prostar 220 Pump

Varian ProStar 330 Photodiode Array

Rheodyne 7725i manual injector with Loop of 10µL

Software Star Chromatography Workstation, version 6.4

PLRP-S Polymer Laboratories Column (8µm, 300Å, 150×4.6mm i.d.)

Jones 7981 Chromatography Column Oven

HPLC CONDITIONS

Column temperature at 40ºC

A) 99% of water/1% of acetonitrile (0.01% of TFA)

B) 99% of acetonitrile/1% of water (0.01% of TFA)

Eluent gradient: 20% to 80% of B in 30min

Flux of 0.6mL/min

Eluent temperature at 40°C

Detection at 210nm

SAMPLE GLIADIN EXTRACTION

Gliadin was extracted with 70% ethanolic solution

15min of Vortex homogenization

Centrifugation at 5000rpm for 10min at 25°C

Filtration with 0.2µm Whatman Nylon Filters

GLIADIN IDENTIFICATION IN SAMPLES

Based on the retention times of the gliadins standard solution

Confirmation based on the UV spectra of each peak detected

STANDARD GLIADIN IDENTIFICATION

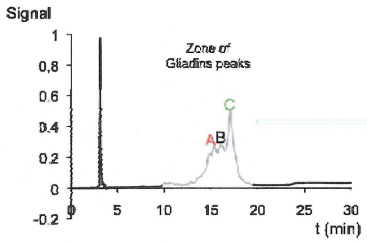

Figure 2. Typical chromatograph of a gliadins Standard solution

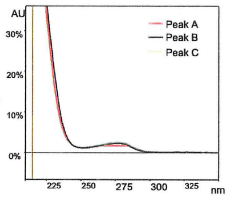

Figure 3. Gliadins UV spectra of HPLC peaks

SAMPLE GLIADIN IDENTIFICATION

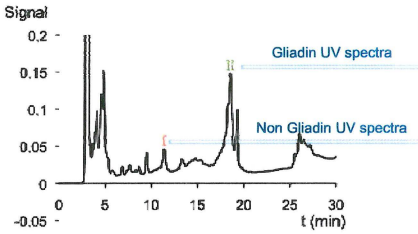

Figure 4. Typical chromatograph of a gliadins sample solution

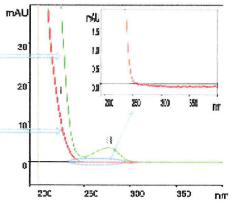

Figure 5. UV spectra of HPLC peaks

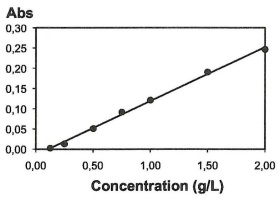

Figure 1. Albumin protein calibration (BSA kit Pierce) at 595nm

GLIADIN HPLC QUANTIFICATION

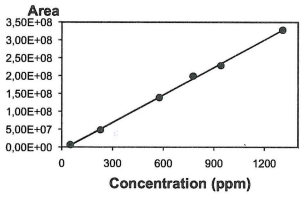

Figure 6. HPLC gliadins calibration measured at 210nm.

RESULTS

- ✧ Linearity: correlation coefficient of 0.9993
- ✧ LD = 46.5 g/L and LQ = 140.9 g/L of gliadins
- ✧ Acceptable repeatability (CV% < 5%)
- ✧ Acceptable intermediate precision (CV% < 5%)
- ✧ Acceptable accuracy (in general, CV% < 5%)
- ✧ Gliadins detected in Flour 3 ("gluten-free")
- ✧ Gliadins quantified in Baby food 3 ("gluten-free")

Table1. Results of gliadins analysis in the food samples

Food samples "with gluten"	
Name	C (mg gliadins/kg sample)
Flour 1	11297
Flour 2	10562
Bread1	3111
Baby food 1	1090
Baby food 2	2982
Waffer 1	d.
Waffer 2	d.
Breakfeast cereals 1	d.

Food samples "gluten free"	
Name	C (mg gliadins/kg sample)
Flour 3	d.
Bread 2	n.d.
Baby food 3	930
Baby food 4	n.d.
Waffer 3	n.d.
Waffer 4	n.d.
Breakfeast cereals 2	n.d.

CONCLUSION

The results showed that "gluten-free" foods must be analysed to determine the gliadins content to ensure that the product label information is correct and, therefore, can be consumed by celiac patients.

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