



P-ISSN2349-8528  
E-ISSN 2321-4902  
IJCS 2016; 4(6): 51-53  
© 2016 JEZS  
Received: 10-09-2016  
Accepted: 11-10-2016

**Siyue Jia**  
College of Medicine, Hebei  
University, Baoding 071000,  
China

**Xiaodong Dong**  
College of Medicine, Hebei  
University, Baoding 071000,  
China

## Recent advances in analytical techniques for the determination of lactulose

**Siyue Jia and Xiaodong Dong**

### Abstract

Lactulose is a disaccharide consisting of one molecule of galactose and one molecule of fructose. It has many applications in medicine and food technology. It is utilized in infant formula and in the prevention and treatment of chronic constipation, portal systemic encephalopathy, and other intestinal and hepatic disorders. Therefore, determination the content of lactulose is very important. In this article the studies of detection methods for lactulose in recent years are reviewed.

**Keywords:** Lactulose, determination, detection, sensor

### 1. Introduction

Lactulose (4-*O*- $\beta$ -D-galactopyranosyl-d-fructose) is a disaccharide firstly produced from lactose by isomerisation in alkaline medium or during heat treatment of milk. Lactulose stimulates the growth of bifidobacteria, for which reason they are referred to as bifidogenic factors in nutrition [1-3]. In addition, lactulose has attracted more and more attention due to its considerable medical interest for the treatment of portal systemic encephalopathy and chronic constipation [4-6]. Nowadays lactulose is widely used in pharmaceutical, nutraceuticals and food industries because of its beneficial effects on human health [7-9]. All of the important applications of lactulose in various fields demonstrate the need for simpler, reliable methods for quantitative determination in biological fluids, pharmaceutical preparations, and dairy products. In this paper, the attributes of different analytical technique for the determination of lactulose in recent years are reviewed.

### 2. Analytical Methods

**2.1. Spectrophotometric method:** High sensitivity, sufficient accuracy, simplicity, speed and the necessity of less expensive apparatus make spectrophotometric method as an attractive method for the determination of lactulose in samples with different matrices such as biological and pharmaceutical samples. The development of the spectrophotometric method is based on the fact that glucose, galactose, and other related sugars present in lactulose solution are aldohexoses, while fructose, the hydrolyzed product of lactulose, is ketohexose, and this difference in functional groups is exploited for the determination of lactulose in pharmaceutical preparations. The investigated method is based on the fact that hydrochloric acid hydrolyzes lactulose into fructose and glucose followed by dehydration and that subsequent reaction of the resulting product with resorcinol gives colored condensation product [10-12].

Khan *et al.* [12] developed a simple spectrophotometric assay for the quantification of lactulose in pharmaceutical preparations. The method was based on hydrolysis of lactulose under acidic conditions. The hydrolyzed product reacted with resorcinol, giving absorption peaks at 398 and 480 nm. Both absorption wavelengths could be used for the determination of lactulose. The limit of detection of lactulose at 398 nm and 480 nm was  $0.075 \mu\text{g mL}^{-1}$  and  $0.65 \mu\text{g mL}^{-1}$ , respectively. The calibration was linear in the range of  $5-25 \mu\text{g mL}^{-1}$ . Analytical conditions were optimized, and the method was validated for analysis of pharmaceutical preparations. The determined amount of lactulose was found to be in good agreement with labeled claims in commercial products. The proposed method was economical, convenient, and suitable for the quantification of lactulose in pharmaceutical preparations.

Zhang *et al.* [13] developed a sensitive and simple spectrophotometric method for the quantification of lactulose without interference from aldoses. The method was based on hydrolysis of lactulose under acidic conditions. The hydrolysed product reacted with cysteine hydrochloride-tryptophan reagent, giving an absorption peak at 518 nm.

**Correspondence**  
**Xiaodong Dong**  
College of Medicine, Hebei  
University, Baoding 071000,  
China

Under the optimized conditions, the absorbance value generated by lactose or galactose was far less than that of lactulose of the same amount, suggesting that the interference from aldoses for determination of lactulose could be neglected. The calibration curve was linear in the range of 5–25  $\mu\text{g mL}^{-1}$  with a correlation coefficient of 0.999. The limit of detection of lactulose at 518 nm was 0.58  $\mu\text{g mL}^{-1}$ . The variation between the results for lactulose (18  $\mu\text{g mL}^{-1}$ ) was 1.02%. These facts revealed that the method could be recommended for the quantitative determination of lactulose in case of syrups, biological fluids or dairy products.

**2.2. HPLC method:** High-performance liquid chromatography (HPLC) is a powerful tool that enables the separation of complex mixtures into individual components, and is a highly sensitive and reproducible analytical technique. In recent years, HPLC has been combined with many sensitive detection techniques and has experienced continuous improvement of stationary phases, which have improved its sensitivity and specificity. HPLC is currently widely used for the analysis of drugs and dosage forms with respect to quality control, quantitative determination of active ingredients and impurities, monitoring drug blood concentration in patients, and bioequivalence assessment [14, 15].

Manzi *et al.* [16] compared four HPLC methods with the aim to develop a more accurate analytical procedure to determine lactulose in milk, together with lactose. The developed method was based on a Carrez precipitation followed by a HPLC separation on two in-series amino-based columns, using  $\text{CH}_3\text{CN}:\text{H}_2\text{O}$  75:25 (v:v) as the mobile phase at 1 ml  $\text{min}^{-1}$  flow rate and a refractive index as the detector. The linearity test for the quantitation of lactulose had been carried out over the range 0.060–1.006 mg  $\text{ml}^{-1}$ , the limit of detection was 0.013 mg  $\text{ml}^{-1}$  and the limit of quantitation was 0.028 mg  $\text{ml}^{-1}$ . The proposed method was simple, cheap and time-saving, and allowed an accurate lactulose–lactose separation, with conventional HPLC equipment.

Nelofar *et al.* [17] developed a simple, swift, sensitive and reproducible HPLC-RI method for the quantification of lactulose and related compounds (fructose, galactose, epilactose and lactose) in oral suspension formulation. The analysis was carried out by using mobile phase (water and acetonitrile 75:25) at the flow rate of 1.0 ml  $\text{min}^{-1}$  on isocratic HPLC-RI system. After manipulating mobile phase composition and mobile phase flow rate, a good separation of five components was achieved within 15 minutes of run time. This study was beneficial to determine the active ingredient as well as the related compounds simultaneously, without using buffer in mobile phase which caused bad resolution and had limitation to analyze on other hyphenated techniques such as LC-MS.

**2.3. Other methods:** In addition to these main approaches mentioned above for lactulose detection, still a few special techniques with high sensitivity have been applied. Pappas *et al.* [18] developed a strategy for direct determination of lactulose in heat-treated milk using diffuse reflectance infrared Fourier transform spectroscopy and partial least squares regression. Fu *et al.* [19] proposed the determination of mannitol and lactulose in urine of colorectal cancer patient by ion-exchange chromatography. Montilla *et al.* [20] developed a reliable gas capillary chromatographic determination of lactulose in dairy samples.

### 3. Conclusions

Lactulose has many applications in medicine and food technology. It usually needs to be measured as a component in complex mixtures containing many other sugars and carbohydrates. In recent years, with the developments of the analytical technologies, the determination of lactulose has become more and more simple, rapid and precise [21, 22]. This review has highlighted the significant developments in rapid and alternative techniques for the detection of lactulose in recent years. We believe the development of lactulose sensors with better sensitivity and specificity, lower cost, simplicity, along with in vivo analytical technique is still the future effort.

### 4. Acknowledgments

The work was supported by the Hebei Provincial Natural Science Foundation of China (No. B2015201161), Medical Engineering Cross Foundation of Hebei University (No. BM201108) and Training Programs of Innovation and Entrepreneurship for Undergraduates of Hebei University (No. 2016061).

### 5. References

- Zhang Z, Yang RJ, Wang H, Ye FY, Zhang S, Hua X. Determination of lactulose in foods: a review of recent research, *Int J Food Sci Technol.* 2010; 45(6):1081-1087.
- Kubica P, Kot-Wasik A, Wasik A, Namiesnik J, Landowski P. Modern approach for determination of lactulose, mannitol and sucrose in human urine using HPLC-MS/MS for the studies of intestinal and upper digestive tract permeability. *J Chromatogr B.* 2012; 907:34-40.
- Silveira MF, Masson LMP, Martins JFP, Alvares TD, Paschoalin VMF, de la Torre CL *et al.* Simultaneous determination of lactulose and lactose in conserved milk by HPLC-RID. *J Chem.* 2015; 2015:185967.
- Gheisar MM, Nyachoti CM, Hancock JD, Kim IH. Effects of lactulose on growth, carcass characteristics, faecal microbiota, and blood constituents in broilers, *Vet Med.* 2016; 61(2):90-96.
- Vovk I, Simonovska B, Kompan L, Prosek M. TLC determination of mannitol and lactulose on amino HPTLC plates, *JPC-J Planar Chromatogr-Mod TLC.* 2003; 16(5):374-376.
- Rentschler E, Kuschel B, Krewinkel M, Claassen W, Gluck C, Jiang B *et al.* Quantification of lactulose and epilactose in the presence of lactose in milk using a dual HPLC analysis, *Food Anal Meth.* 2016; 9(8):2210-2222.
- Chae JP, Pajarillo EAB, Oh JK, Kim H, Kang DK. Revealing the combined effects of lactulose and probiotic enterococci on the swine faecal microbiota using 454 pyrosequencing, *Microb Biotechnol.* 2016; 9(4):486-495.
- Seo YH, Sung M, Han JI. Lactulose production from cheese whey using recyclable catalyst ammonium carbonate, *Food Chem.* 2016; 197:664-669.
- Sunny JK, Garcia CJ, McCallum RW. Interpreting the lactulose breath test for the diagnosis of small intestinal bacterial overgrowth. *Am J Med Sci.* 2016; 351(3):229-232.
- Panesar PS, Kumari S. Lactulose: Production, purification and potential applications, *Biotechnol Adv.* 2011; 29(6):940-948.
- Khan MA, Jan MR, Shah J, Iqbal Z, Khan H. Spectrophotometric determination of lactulose in pharmaceutical preparations using acid hydrolysis, *Am Lab.* 2004; 36(14):41-44.

12. Khan MA, Iqbal Z, Jan MR, Shah J, Ahmad W, Haq ZU *et al.* A spectrophotometric method for quantitative determination of lactulose in pharmaceutical preparations, *J Anal Chem.* 2006; 61(1):32-36.
13. Zhang Z, Wang H, Yang RJ, Jiang XY. A novel spectrophotometric method for quantitative determination of lactulose in food industries. *Int J Food Sci Technol.* 2010; 45(2):258-264.
14. Ye NS, Gao T, Li J. Hollow fiber-supported graphene oxide molecularly imprinted polymers for the determination of dopamine using HPLC-PDA, *Anal Methods.* 2014; 6(18):7518-7524.
15. Capone DL, Ristic R, Pardon KH, Jeffery DW. Simple quantitative determination of potent thiols at ultratrace levels in wine by derivatization and high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) analysis, *Anal Chem.* 2015; 87(2):1226-1231.
16. Manzi P, Pizzoferrato L. HPLC determination of lactulose in heat treated milk, *Food Bioprocess Technol.* 2013; 6(3):851-857.
17. Nelofar A, Laghari AH, Yasmin A. Validated HPLC-RI method for the determination of lactulose and its process related impurities in syrup. *Indian J Pharm Sci.* 2010; 72(2):255-258.
18. Pappas CS, Sakkas L, Moschopoulou E, Moatsou G. Direct determination of lactulose in heat-treated milk using diffuse reflectance infrared Fourier transform spectroscopy and partial least squares regression. *Int J Dairy Technol.* 2015; 68(3):448-453.
19. Fu XL, Zhou RY, Pan GW. Determination of mannitol and lactulose in urine of colorectal cancer patient by ion-exchange chromatography. *Chin J Anal Chem.* 2012; 40(4):608-611.
20. Montilla A, Moreno FJ, Olano A. A reliable gas capillary chromatographic determination of lactulose in dairy samples, *Chromatographia.* 2005; 62(5-6):311-314.
21. Linsalata M, D'Attoma B, Orlando A, Guerra V, Russo F. Comparison of an enzymatic assay with liquid chromatography-pulsed amperometric detection for the determination of lactulose and mannitol in urine of healthy subjects and patients with active celiac disease, *Clin Chem Lab Med.* 2014; 52(4):E61-E64.
22. Luzzana M, Agnellini D, Cremonesi P, Caramenti G, De Vita S. Milk lactose and lactulose determination by the differential pH technique, *Lait.* 2003; 83(5):409-416.