Recent advances in analytical techniques for the determination of lactulose

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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-β-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 μg mL⁻¹ and 0.65 μg mL⁻¹, respectively. The calibration was linear in the range of 5–25 μg 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.
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 CH₃CN:H₂O 75:25 (v:v) as the mobile phase at 1 ml 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 ml⁻¹ and the limit of quantitation was 0.028 mg 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 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.

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5. References