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Identification, method development, validation, and characterization of Aza sugars by an ion-chromatography, high-resolution mass spectrometer, and LC-MS/MS

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Abstract

Background: Aza sugars are organi c sugars having nitrogen containing polyhydroxyl sugar molecules. These molecules are active pharmaceutical ingredients; these are not well separated and eluted early in the HPLC and UPLC columns due to high polar nature. Aza sugars are having high conductivity hence the ion chromatography validated method has been established for the castanospermin and celgosivir along with its degradation studies (impurities).

Methods: An ion chromatography with conductivity detector-cation column was used to determine the assay of castanospermin and celgosivir as in the form of bulk active pharmaceutical ingredients. The degradation impurities were identified and characterized by using the UPLC-TOF and the LCMS/MS techniques.

Results: An ion chromatography method was developed and determined the assay for castanospermin and celgosivir as in the form of bulk active pharmaceutical ingredients with the specificity of the miglitol and 1-deoxynojirimycin. Validation was performed for assay of the castanospermin and celgosivir. The method precision %RSD results at 0.25 mg/mL concentration of castanospermin and celgosivir were 1.1 and 0.7 respectively. The linearity was performed from 25 to 200% (w.r.t 0.25 mg/mL); the results were 1.000 and 0.999 coefficient for the castanospermin and celgosivir respectively. The recovery studies, robustness, ruggedness, and solution stability results were within the acceptance limits of the ICHQ2 (R1) guidelines. The stress study for the castanospermin and celgosivir active pharmaceutical ingredients was performed by using 0.5N HCl solution, 0.5N NaOH solution, 3.0% H202 solution, UV-visible and the thermal conditions. The castanospermin was degraded as 20.8% of n-oxide impurity, and celgosivir was degraded as 10.0% n-oxide impurity under 3.0% H202 solution. In base degradation, the celgosivir was back converted completely to castanospermin. These n-oxide impurities were identified and characterized by using UPLC-TOF and LCMS/MS techniques after collection from the ion chromatography. Castanospermin and celgosivir are stable in remaining stress conditions.

Conclusions: From the present study, it was found that robust analytical ion chromatography technique is used for the determination of assay in Aza sugar, especially assay for the castanospermin and celgosivir with minimum usage of test sample 0.25 mg/mL and used green chemistry solvents. The study also explains that the unique degradation of castanospermin and celgosivir under oxidative and base hydrolysis, Oxidative degradation impurities were identified and characterized as n-oxides of its respective castanospermin and celgosivir active pharmaceutical ingredients by using HRMS and LC-MS/MS.

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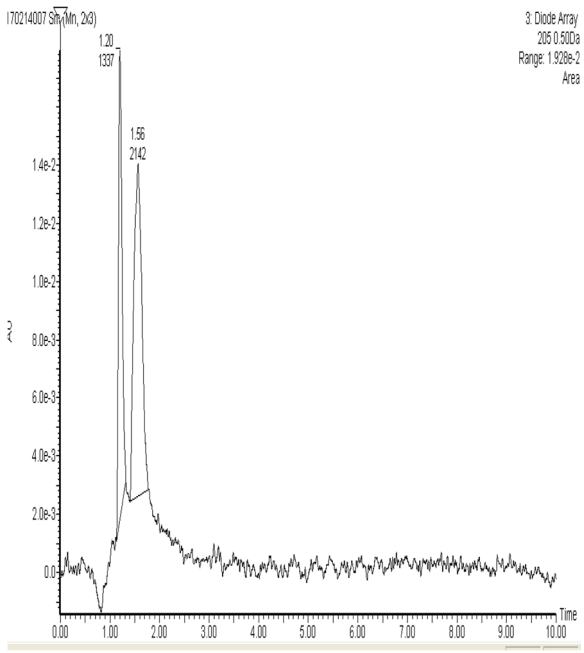


Fig. 1 UPLC method chromatogram of Aza sugars

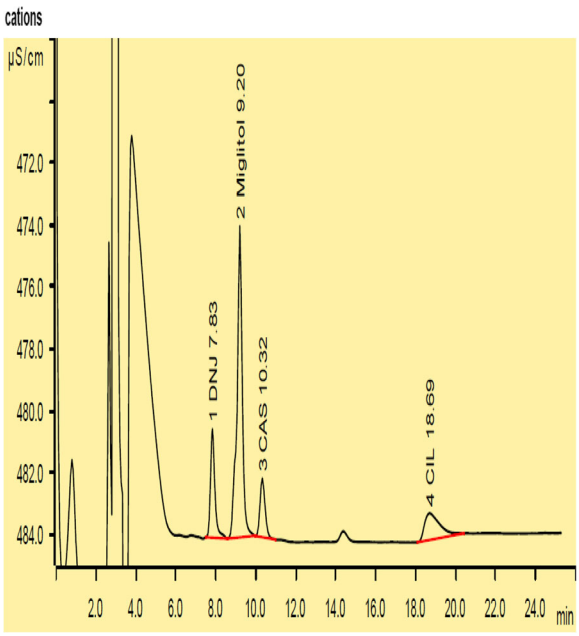


Fig. 3 Blend chromatograms of selected Aza sugars with Metrosep C4 250/4.0 column

Background

1-Deoxynojirimycin, miglitol, castanospermin, and celgosivir are some of the selected Aza sugars, which are now used for the treatment of diabetes, dengue, and hepatitis C virus (HCV) diseases respectively. Celgosivir is in development by Migenix for the treatment of HCV infection; it is an oral prodrug of the natural product. Castanospermin inhibits alpha-glucosidase, an enzyme that plays a critical role in viral maturation by initiating the processing of the N-linked oligosaccharides of viral envelope glycoproteins. Celgosivir is well

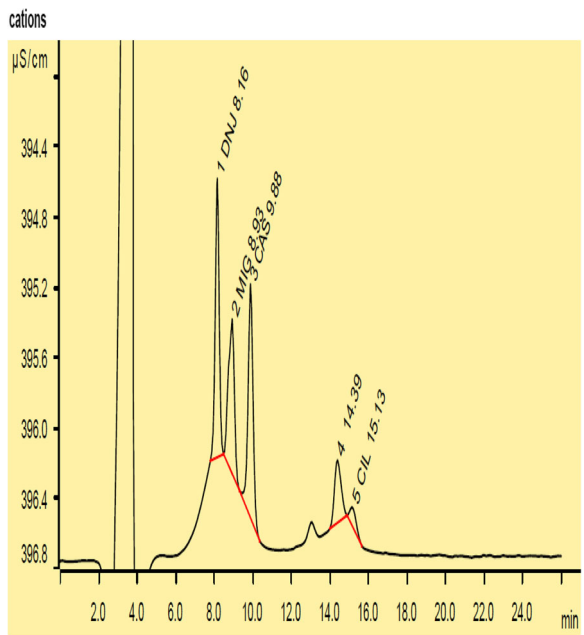


Fig. 2 Development of Aza sugars (1-deoxynojirimycin, miglitol, castanospermin, and celgosivir) with universal cation column in ion chromatography

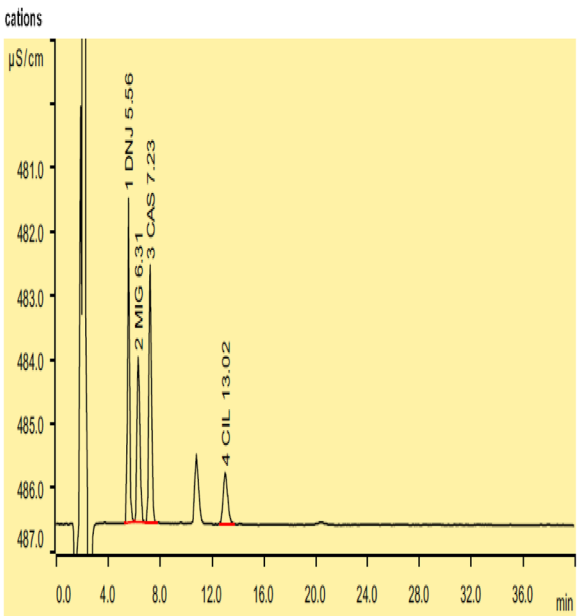


Fig. 4 Blend chromatograms of selected Aza sugars (1-deoxynojirimycin, miglitol, castanospermin, and celgosivir) with Metrosep C4 150/4.0 column

absorbed in vitro and in vivo and rapidly gets converted to castanospermin. Celgosivir has a novel mechanism of action and demonstrates broad antiviral activity in vitro. Celgosivir is not efficient as a monotherapy for the treatment of HCV but has a synergistic effect in combination with pegylated interferon alfa-2b plus ribavirin, both in vitro and in phase II clinical trials that last up to 1 year in patients with chronic HCV infection. Celgosivir is a 6-0-butanoyl ester derivative of castanospermin, a compound derived from the Australian chestnut with activity against hepatitis C virus. Celgosivir rapidly converts to castanospermin in the body, where it is a potent inhibitor of alpha-glucosidase (Belley et al. 2013), a host enzyme required for viral assembly, release, and infectivity. Castanospermin is an indolizidine alkaloid first isolated from the seeds of *Castanospermum australe*. It is a potent inhibitor of some glucosidase enzymes and has antiviral activity in vitro and in mouse models. 1-Deoxynoijirimycin is also called as duvoglustat or moranolin. It is an inhibitor; it is mostly present in the mulberry leaves and also present by brewing little quantities from the (herbal tea) of the mulberry leaves. Miglitol is an oral anti-diabetic drug; it is acting as an inhibitor, the ability to break down complex carbohydrates into glucose. It is used in

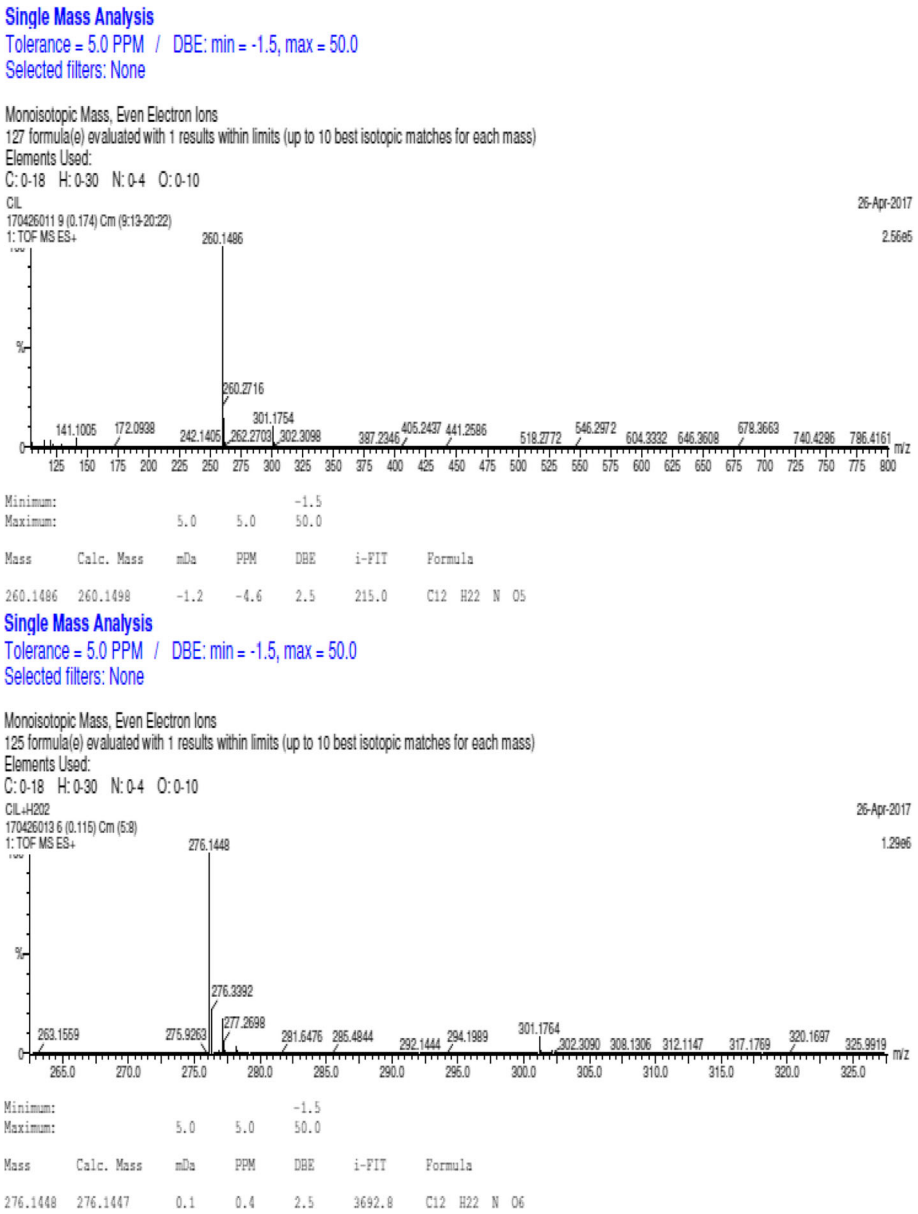


Fig. 5 HR-MS spectrum of celgosivir and its n-oxide impurity

diabetes mellitus type 2 for establishing greater glycemic control which prevents the digestion of disaccharides, oligosaccharides, and polysaccharides into monosaccharides which can be absorbed by the body.

As per the knowledge of the author, there was no analytical method proposed for Aza sugars with selection. Many HPLC methods were there for the determination of miglitol in drug products and drug substances (Balakumaran et al. 2016; Dhole et al. 2012; Chittora et al. 2009). Some of the HPLC methods were available for the determination of miglitol content in human plasma. The liquid chromatography is an available method for the identification and quantification of miglitol in drug substance, drug product, and human plasma (Li et al. 2007; Nirogi et al. 2006; Wang et al. 2005). The unique separation techniques like electrophoresis, kinetic study instruments are used for the determination of miglitol in bulk drug substance (Cahours et al. 2002; Ibrahim et al. 2007). Similarly, many HPLC, UPLC, and ion chromatographic methods are available for the determination of 1-deoxynojirimycin as bulk and in mulberry plants (Japan Intl. Research Center for Agricultural Sciences et al. 2010; Rudraprasad Reddy et al. 2014; Kimura et al. 2004; Rajana et al. 2016).

The castanospermin and celgosivir are well biological active drugs for curing the different diseases (Budavari et al. 1989; Hohenschutz et al. 1981; Saul et al. 1985; Whitby et al. 2005; Durantel et al. 2009; Whitby et al. 2004); these are highly polar polyhydroxyl Aza compounds. As per the author, no best analytical method can be determined for castanospermin and celgosivir as single method. The present study is novel and also capable of characterizing the process impurities and degradation impurities for castanospermin and celgosivir as active pharmaceutical ingredients (International Conference on Harmonization (ICH) 2005; 1996; USP39 2016; Council of Europe 2015). The other polyhydroxyl Aza

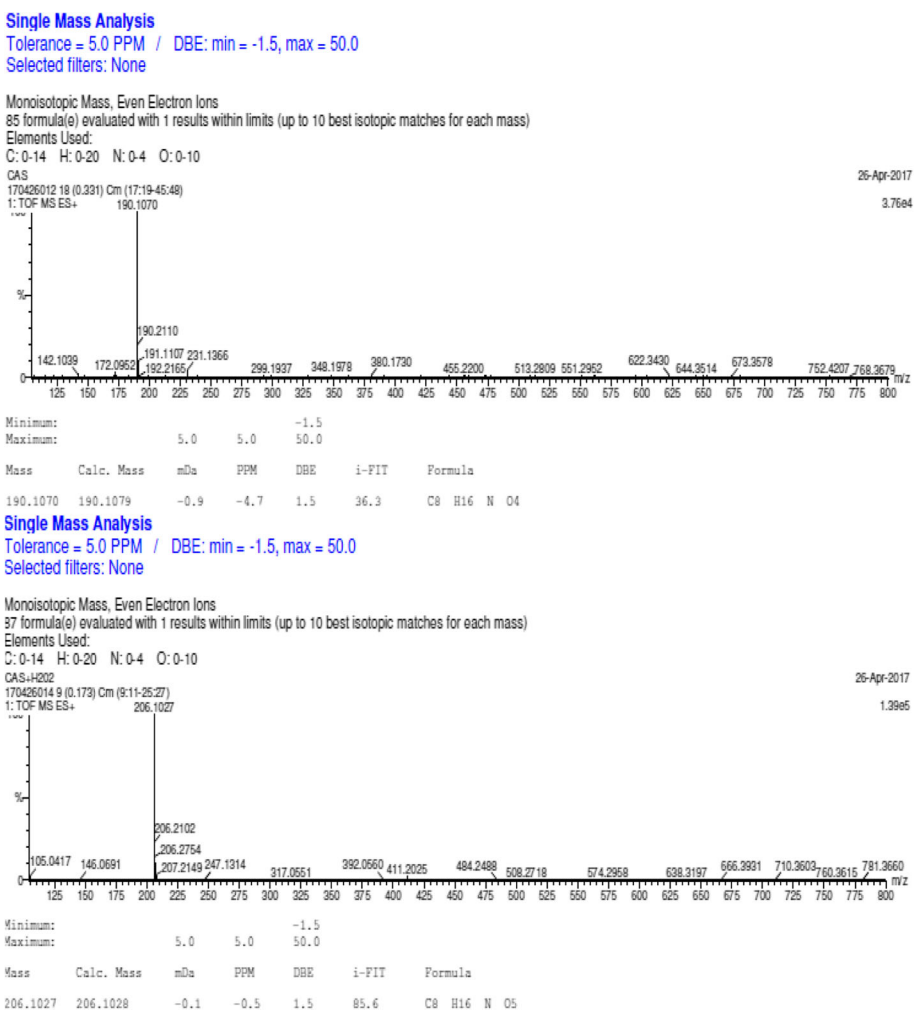


Fig. 6 HR-MS spectrum of castanospermin and its n-oxide impurity

sugars are characterized by high-resolution mass spectrometry and LC-MS/MS.

Methods

Chemicals and reagents

1-Deoxynojirimycin, miglitol, castanospermin, and celgosivir are synthesized and purified from the process research and development of Custom Pharmaceutical Services of Dr. Reddy's Laboratories Limited. Analytical reagent grade tartaric acid, pyridine-2,6-dicarboxylic acid, sodium hydroxide, hydrochloric acid, and hydrogen peroxide were purchased from Rankem (Mumbai, India). Millipore Milli Q purification system purchased from Bangalore, India. Photo stability chamber is with UV meter and master digital lux meter purchased from Mack PharmaTech, India. Vacuum oven was purchased from Cintex, India.

Ion chromatography—optimization of chromatographic conditions

Metrohm ion chromatography instrument with MagIC Net 3.2 software was used for the total study. The ion chromatography system was bought from Metrohm, Herisau, Switzerland. The ion chromatography system is equipped with 858 professional sample processor, 818 IC pump, sampling injector with a 20-μL loop, 882 compact IC plus with a cation suppressor, and a conductivity detector. Analysis of Aza sugars have been performed from the output signal, monitored, and processed using the MagIC Net 3.2 version software on a Compaq computer (Digital Equipment Corp.). Dilutions were performed with Hamilton Precision Pipettes (Bondaiz, Switzerland). Chromatographic separation was performed on Metrosep C4 150/4.0 column at ambient temperature

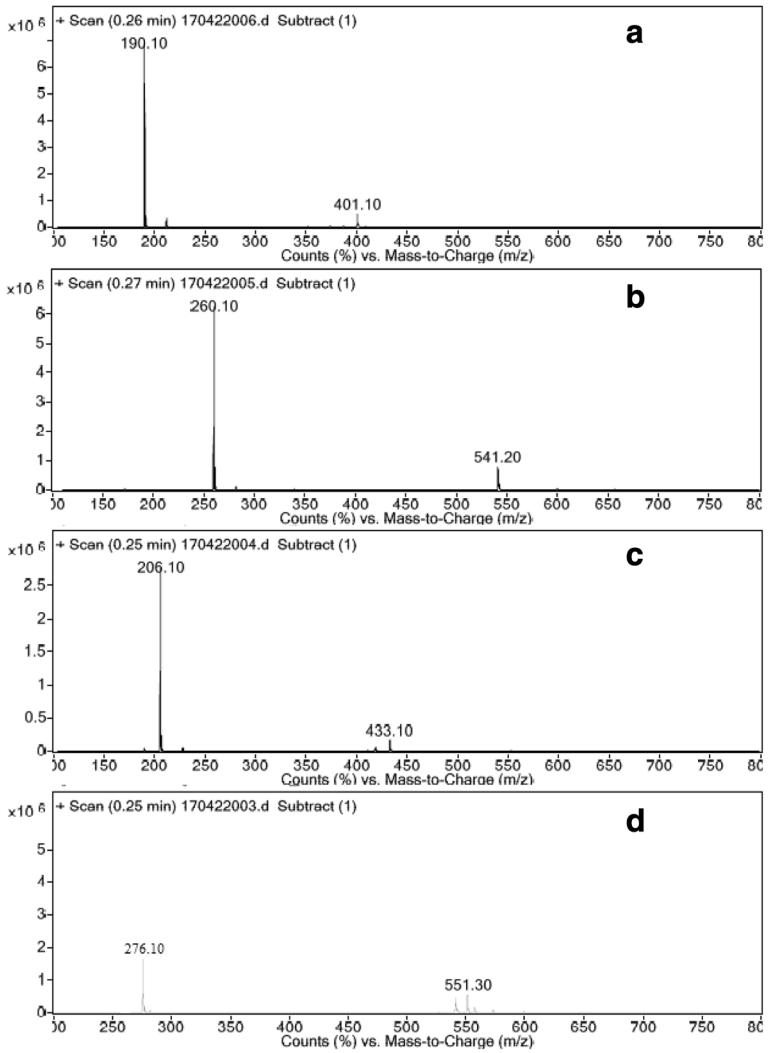


Fig. 7 Scan mode m/z of **a** celgosivir, **b** castanospermin, **c** n-oxide impurity of celgosivir, and **d** n-oxide impurity of castanospermin

with 4 mM tartaric acid and 1.5 mM pyridine-2,6-dicarboxylic acid mixed buffer and acetone (%*v*/*v* 90:10). The analysis was performed at 0.7 mL flow and 20-injection volume loop with conductivity detector.

High-resolution mass spectrometry—optimization of mass spectrometry conditions

Water AQUATY UPLC-TOF instrument with LCT Premier XE Mass Lynx TM software was used for the identification, characterization of process impurities, and degradation impurities of castanospermin and celgosivir. The mobile phase used for this study was 10 mM ammonium acetate and acetonitrile (%*v*/*v* 50:50). The injection volume was 2.0 μL on Acquity TM binary solvent manager with waters TOF with LCT Premier XE.

LC-MS/MS—optimization of mass spectrometry conditions

LC-MS/MS study of castanospermin and celgosivir and their degradation impurities were recorded with LC-MS G6410 QqQ instrument (Agilent Technologies). The used condition was source temperatures at 325 °C, gas flow was 10 L/min, capillary voltage was 4000 V, and the mass range was 100–800 a.m.u. The heater temperatures were 100 °C for the both MS1 and MS2 heaters. The EMV was 10 for mass spectrometer. The collision gas was nitrogen for MS/MS of APIs and degradation impurities. The different

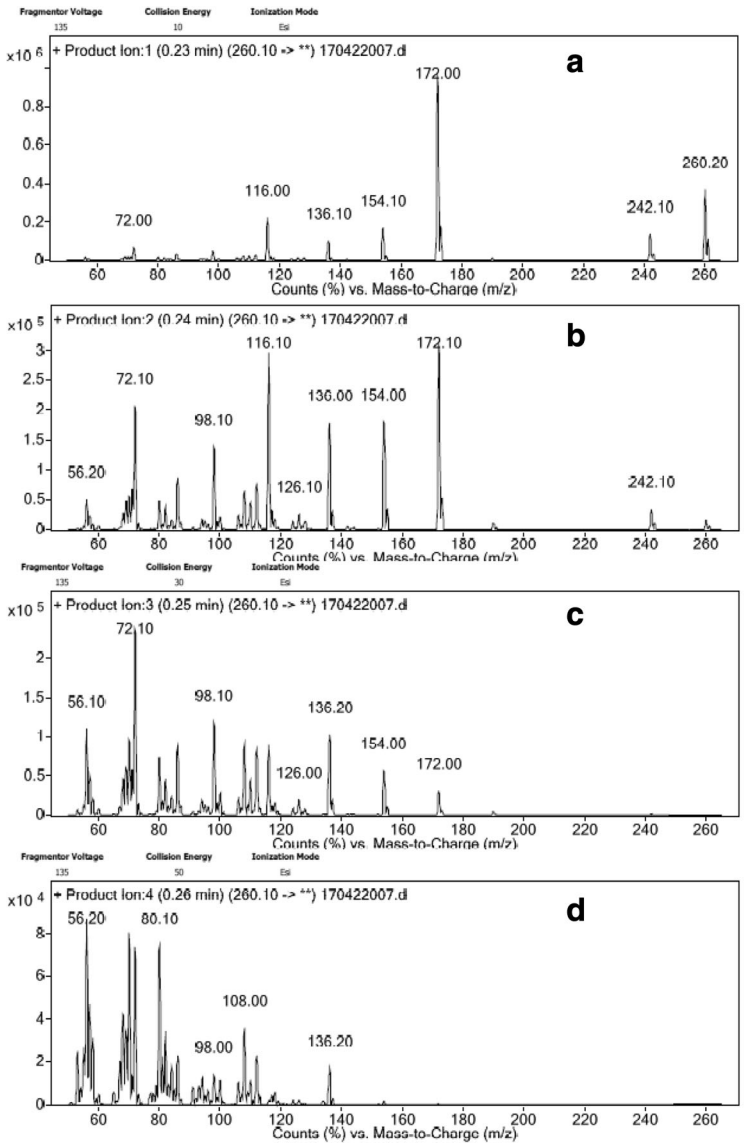


Fig. 8 MS/MS of celgosivir at **a** 10 eV collision energy, **b** 20 eV collision energy, **c** 30 eV collision energy, and **d** 50 eV collision energy

collision energies were used for the complete study of unknown degradation impurities. The LC condition used were (% *v*/*v*) 25:75: 0.1% formic acid in water and 0.1% formic acid in acetonitrile, the injection volume for the present study of LC-MS/MS was 5.0 μL, and diluent was (%*v*/*v*: 10:90): acetonitrile and water. The MS/MS study was performed by using the flow 0.5 mL/min, run time 1.0 min, 0.25 mg/mL sample concentration and union.

Standard and sample solution preparation

The Aza sugars' standard solution was prepared by weighing 25 mg of each catanospermin and celgosivir and transferring separately in to 100 mL volumetric flask containing 25 mL of water. Make up to the mark with diluent and cyclomix standards with vortex cyclomixer. The solution was used for the ion chromatography injection.

Specificity solution

The specificity of the ion chromatography method was demonstrated with 1-deoxynojirimycin and miglitol along with castanospermin and celgosivir. The specificity standard solution used for this present study was weighed and transferred about each 25 mg of 1-deoxynojirimycin, miglitol, castanospermin, and celgosivir in a 100-mL volumetric flask contain 25 mL of water and make up to the mark with diluent, cyclomix standards with vortex cyclomixer.

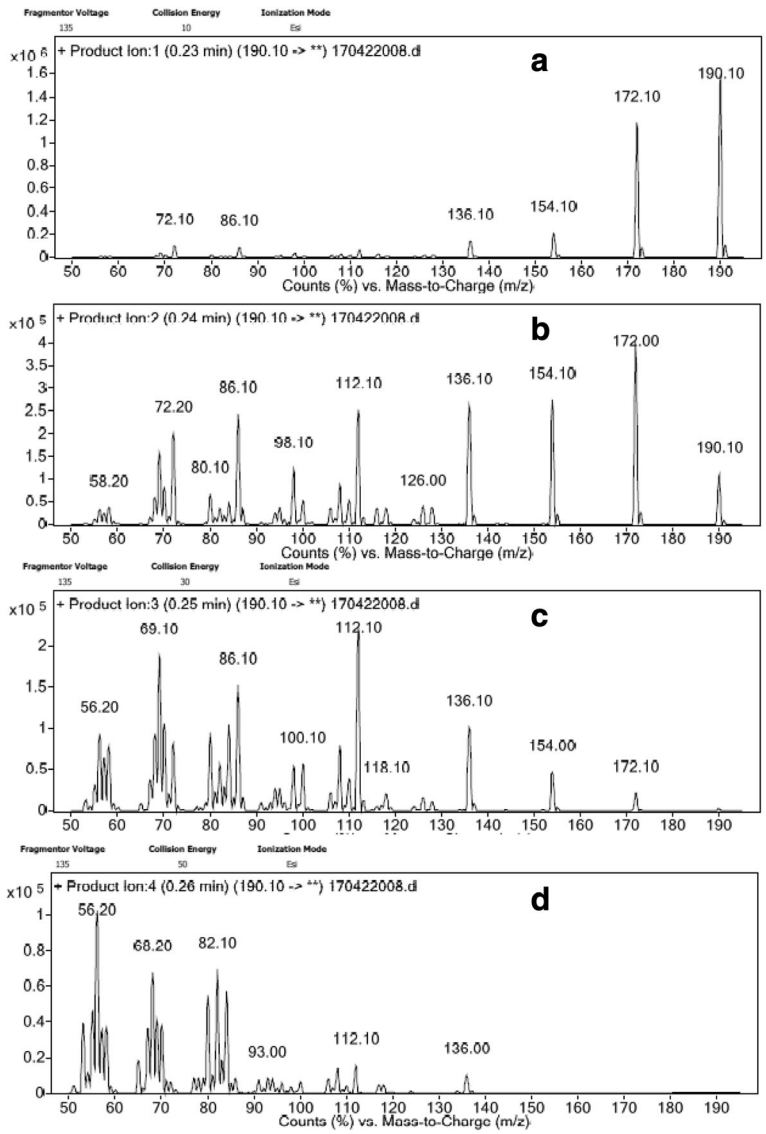


Fig. 9 MS/MS of castanospermin at **a** 10 eV collision energy, **b** 20 eV collision energy, **c** 30 eV collision energy, and **d** 50 eV collision energy

Method validation of castanospermin and celgosivir by ion chromatography

The optimized ion chromatography method was validated as per the ICH guidelines and validation of compendial procedures as per USP general chapter 1225. The validation parameters for the ion chromatographic method were specificity, precision, accuracy, linearity, robustness, and ruggedness.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. The specificity was done for the no interference with other matrix and degradants along with studied active pharmaceutical ingredients. The degradation study was done by acid and base hydrolysis and oxidation; the degradation impurities were identified by using high-resolution mass spectrometry and LC-MS/MS.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements

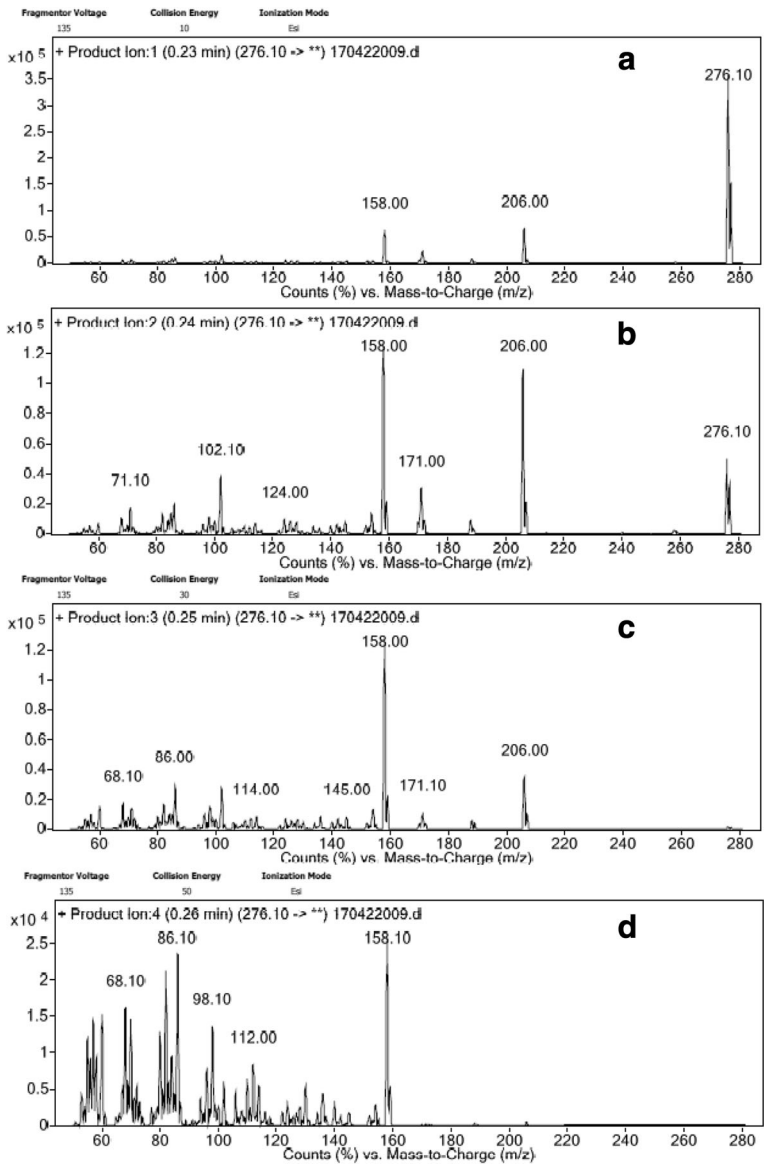


Fig. 10 MS/MS of n-oxide impurity of celgosivir at **a** 10 eV collision energy, **b** 20 eV collision energy, **c** 30 eV collision energy, and **d** 50 eV collision energy

obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility. The precision was studied for the system suitability test and method precision, 50% level and 150% level with respect to 100% level. Average, standard deviation, and variance for the six preparations were calculated for each parameter.

Accuracy

It is also called trueness. The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy at 50, 100, and 150% levels were calculated, and average recovery was calculated at all levels.

Linearity

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. The linearity of the ion chromatography method study for the 25 to 200% (w.r.t test concentration 0.25 mg/mL) has been studied, and correlation coefficient was calculated for both castanospermin and celgosivir.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. To test the robustness parameter, the precision and accuracy were done by changing flow and buffer concentration, and the %RSD were calculated.

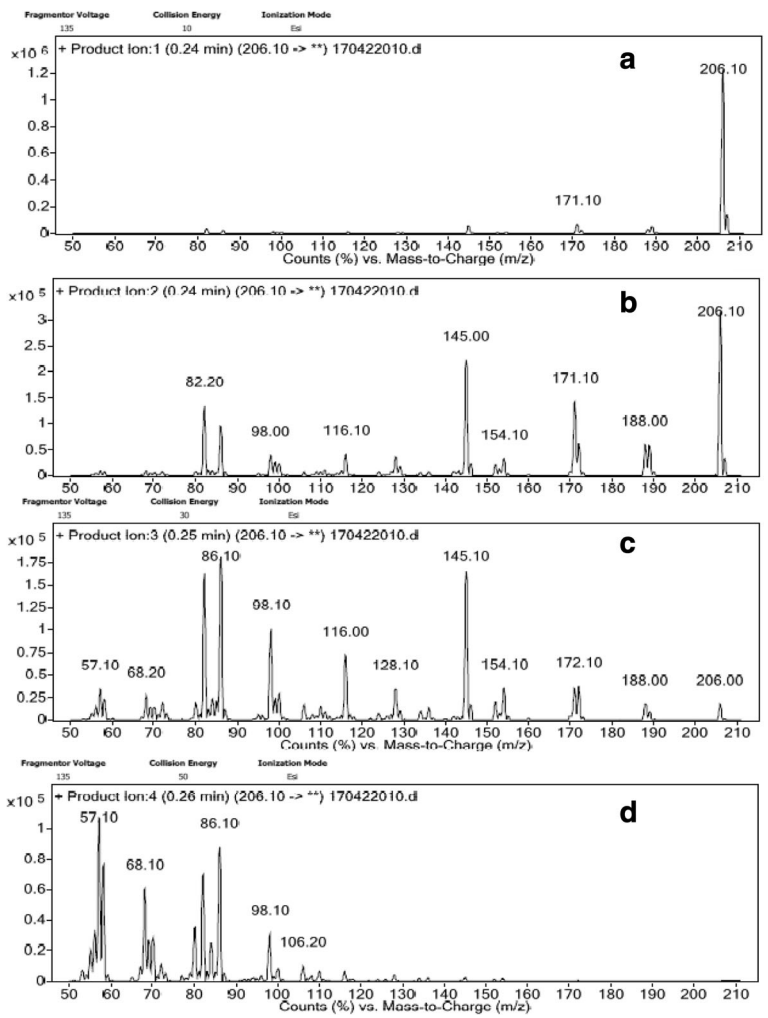


Fig. 11 MS/MS of n-oxide impurity of castanospermin at **a** 10 eV collision energy, **b** 20 eV collision energy, **c** 30 eV collision energy, and **d** 50 eV collision energy

Ruggedness

The ruggedness parameter is consisting of intermediate precision and repeatability. The intermediate precision was performed by changing the different column and different instrument in the same lab, the study was extended in different laboratories, and the results were calculated and reported the %RSD for two labs.

Solution and mobile phase stability

Solution stability is the stability for the same solution of drug substance at different intervals of time with optimized conditions. Mobile phase is stable at fresh preparation in different intervals of time with the same mobile phase along with other method parameters.

Results and discussion

Ion chromatography—method development and optimization

The objective of the ion chromatographic method is to develop the novel isocratic method for assay determination in Aza sugars having polyhydroxyl compounds such as 1-deoxynojirimycin, miglitol, castanospermin, and celgosivir. The degradation study of castanospermin and celgosivir has been carried out by ion chromatography and characterization by high-resolution mass spectrometry and LC-MS/MS. In initial stage, analytical method development has been started with ultra-performance liquid chromatography. The peaks of castanospermin and celgosivir were eluted early, retained in C18 column with high-concentration potassium buffer with sample concentration of 10 mg/mL, and they are less UV active in Fig. 1. Henceforth, the analytical method development has been started by using ion chromatography technique with cation column, conductivity detector, and ion chromatography compatible buffers. In ion chromatography, the initial development was started by using the universal column with 3 mM nitric acid buffer and conductivity detector; the Aza sugars were eluted as broad peaks with co-elution (Fig. 2). The universal column has been replaced with Metrosep C4 150/4.0 column with 3.0 mM nitric acid buffer: Acetone: 90:10 (%*v*/*v*), the peaks of 1-deoxynojirimycin, miglitol, and castanospermin were separated but not resolved properly. Instead of Metrosep C4 150/4.0, the long column (Metrosep C4 250/4.0) with the same conditions like the previous trials were used for resolution of 1-deoxynojirimycin, miglitol, and castanospermin but the profile of all peaks came late and peaks become broad and no good resolution was observed between the 1-deoxynojirimycin, miglitol, and castanospermin (Fig. 3). The weak organic buffer such as 4 mM tartaric acid, 0.75 mM pyridine-2,6-dicarboxylic acid mixed buffer and acetonitrile used (%*v*/*v* 90:10), but the resolution between 1-deoxynojirimycin and miglitol was less. The study was continued with acetone instead of the acetonitrile; the

Table 1 m/z values of castanospermin, celgosivir, and its oxidative impurities

Name of product	Molecular ion (m/z)	Collison energy (eV)	Fragment ions (m/z)
Celgosivir	260.10	0	ND
	260.10	10	242,172,154,136,116,72
	260.10	20	242,172,154,136,126,116,98,80,72,56
	260.10	30	172,154,136,126,116,108,98,80,72,56
	260.10	50	136,126,116,108,98,80,72,56
Castanospermin	190.10	0	ND
	190.10	10	172,154,136,86,72
	190.10	20	172,154,136,126,112,98,86,80,72,58
	190.10	30	172,154,136,118,112,100,86,69,56
	260.10	50	136,112,93,82,68,56
Celgosivir	276.10	0	ND
(Oxidative impurity)	276.10	10	206,158
	276.10	20	206,171,158,124,102,71
	276.10	30	206,171,158,145,114,86,68
	276.10	50	158,112,98,86,68
Castanospermin	206.10	0	ND
(Oxidative impurity)	206.10	10	172
	206.10	20	188,172,154,145,116,98,82
	206.10	30	188,172,154,145,128,116,98,86,68,57
	206.10	50	106,98,86,68,57

ND Not detected

resolution increased more than acetonitrile trial, then the concentration of tartaric acid increased from 4 to 5 mM the all peaks came early with same conditions of above trials, all the peaks eluted early than previous profile and observed the resolution also less. Finally, 4 mM tartaric acid and 1.5 mM pyridine-2,6-dicarboxylic acid mixed buffer and acetone (%*v*/*v* 90:10) was used with 0.7 mL flow and 20-injection volume loop with conductivity detector and Metrosep C4 150/4.0 column, and analysis was done at ambient temperature (Fig. 4). The profile of all selected Aza sugars and degradation impurities was well resolved. The final method was validated against the ICHQ2 (R1).

High-resolution mass spectrometry—method development, optimization, identification, and characterization of unknown degradation impurities

The development of the ESI method was done with DIP mass and LC-MS; the mass of 1-deoxynojirimycin, miglitol, castanospermin, and celgosivir were observed with proton adduct, i.e., [M + H] + and the unknown degradation peaks of castanospermin and celgosivir were identified as n-oxides of castanospermin and celgosivir. The mobile phase used for this study was 10 mM ammonium acetate and acetonitrile (%*v*/*v* 50:50). The injection volume was 2.0 μL on Acquity ™ binary solvent manager with waters TOF with LCT Premier XE. Instrument parameters were polarity, ES+, Analyser, W-Mode, Capillary (V) 1500.0, sample cone (V): 20.0, desolvation temp (°C):250.0, source temp (°C):120.0, cone gas flow: 25.0, desolvation flow: 250.0, ion guide one: 5.0, aperture 1 voltage: 5.0. ion energy(V): 29.0, aperture 2 voltage: 1.5, Hex pole DC voltage:5.0, aperture 3 voltage: 10.0, acceleration (V): 90.0, Y focus (V): 68.0, steering (V): 2.1, tube lens (V): 30.0, attenuated: Z, focus (V): 85.9, TOF fight tube(V): 7200.0, reflectron (V): 1800.0, pusher (V): 900.0, pusher offset voltage: 0.9, puller voltage: 752.0, puller offset (V): 0.0, MCP detector (V): 2600, PusherCycleTimeAuto(60), PusherFrequency:16666.67, pusherwidthe: 400, Centroidthreshold:

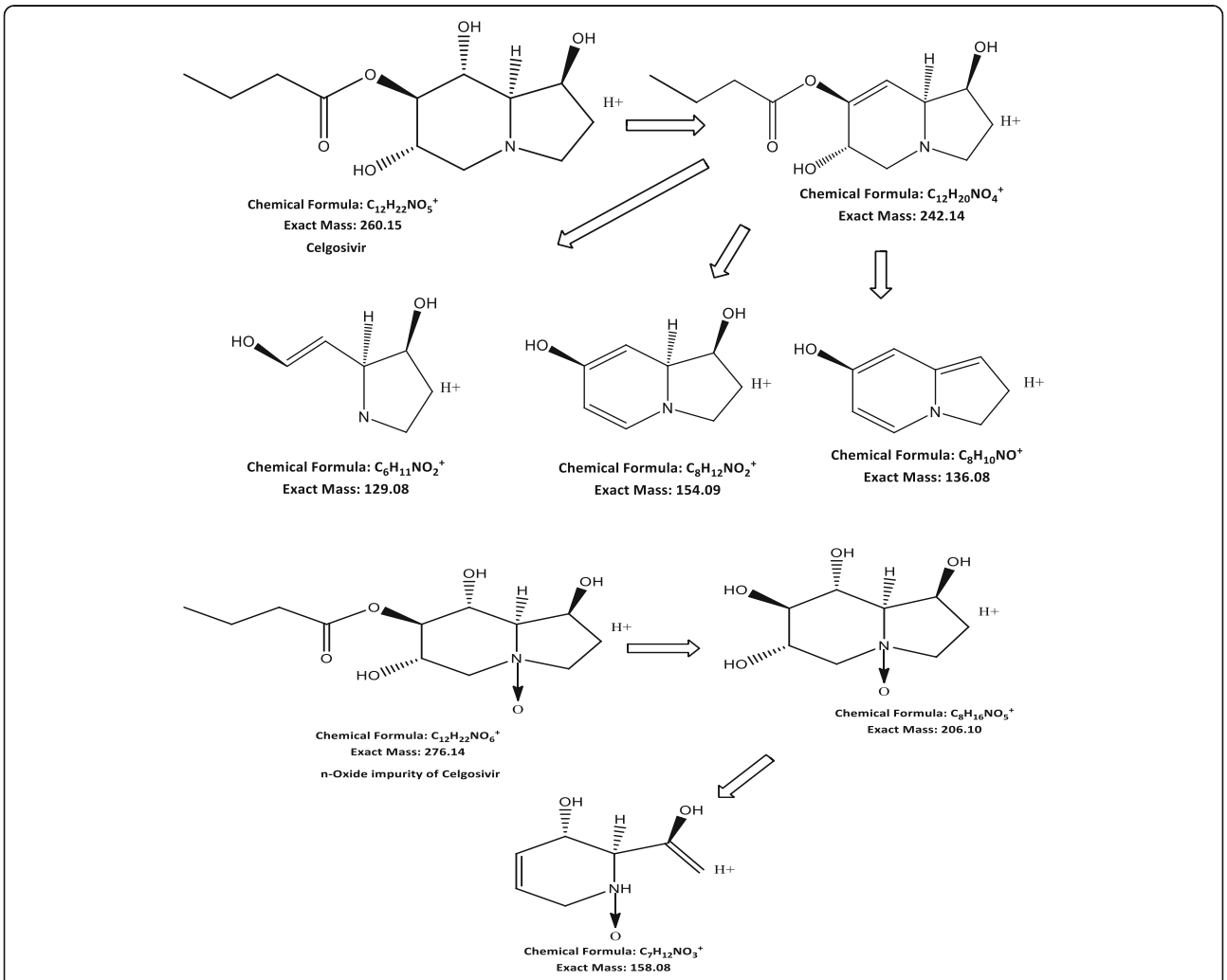


Fig. 12 Probable fragmentation pattern of celgosivir and n-oxide impurity

1.0, Minpoints: 2.0, Npmultiplier: 0.70, resolution: 9533.0, Lteff: 2225.0000, Veff: 7193.7285, trigger threshold (Mv): 600.0000. Signal Threshold (mV) 60.0000, Data Threshold: 0.0000, DXC Temperature: 25.0, Scans in function: 277, Cycle time (secs): 0.410, Scan duration (secs):0.40, Inter scan delay (s):0.01, retention window (mins): 0.000 to 10.000, Ionization mode: ES+, function type: TOF MS, Mass range: 105 to 1000 (Figs. 6 and 7).

The formulae of all selected Aza sugars and its degradation impurities were matching with theoretical mass number with less than 5.0 ppm error value. The celgosivir molecular formula (C12H22NO5: [M + H]) accuracy error ppm was − 4.6 ppm, and the celgosivir peroxide degradation impurity molecular formula (C12H22NO6: [M + H]) was 0.4 ppm with 2.5 DBE. Hence, the celgosivir peroxide degradation impurity was proposed as n-oxide impurity of celgosivir (Fig. 5). Similarly, the castanospermin molecular formula (C18H16NO4: [M + H]) accuracy error ppm was − 4.7 ppm and the castanospermin peroxide degradation impurity molecular formula (C18H16NO5: [M + H]) was − 0.5 ppm with 1.5 DBE. Hence, the castanospermin peroxide degradation impurity was proposed as n-oxide impurity of castanospermin (Fig. 6).

LC-MS/MS—method development, optimization, identification, and characterization of unknown degradation impurities

The MS/MS spectra of celgosivir and castanospermin and their peroxide degradation impurities are shown in Figs. 7 and 8. At lower collision energies, i.e., 10 and 20 eV, the molecular ion peak at 260 and 190 m/z was observed for celgosivir and castanospermin respectively (Figs. 8, 9, and 10). At higher collision energies, i.e., 30 and 50 eV, the butanone group in celgosivir and –OH group in castanospermin were eliminated and observed 206 m/z for celgosivir and 172 m/z for castanospermin, further fragmented to 158 m/z for the celgosivir (Fig. 8) and 154 m/z for castanospermin (Fig. 9). HR-MS data supported the fragmentation profile of celgosivir and castanospermin (Figs. 6 and 7). At the lower collision energies, the stable molecular ion

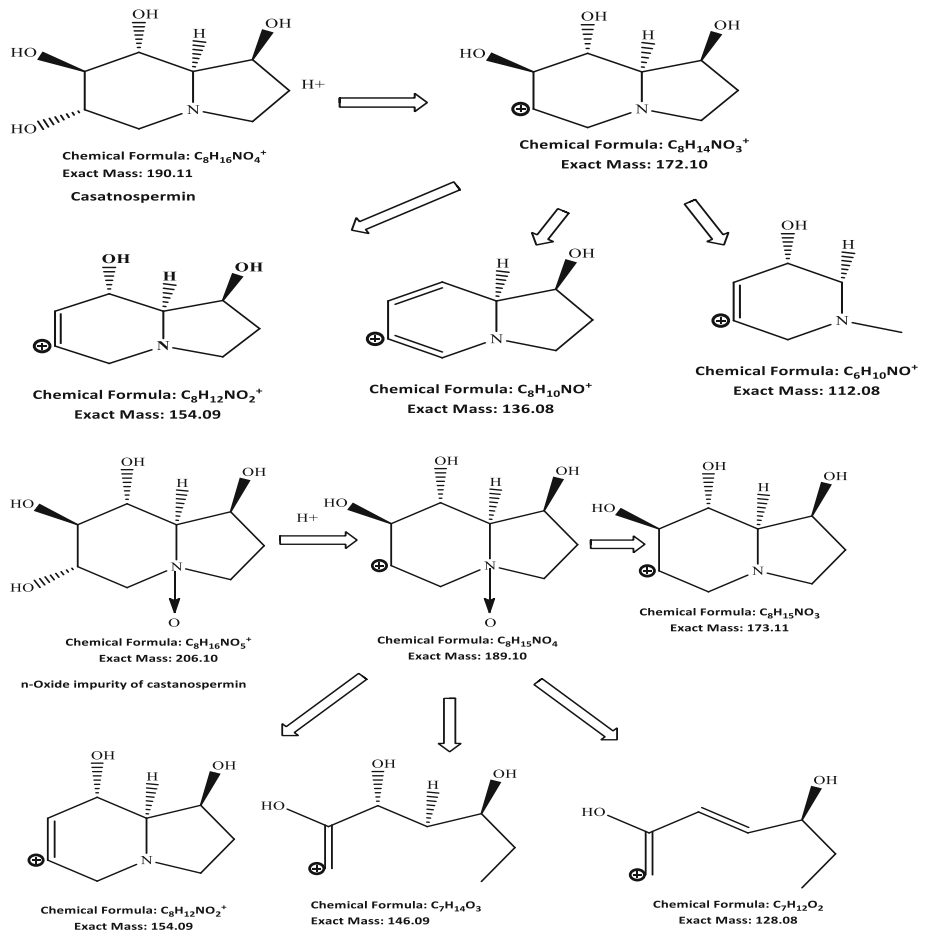
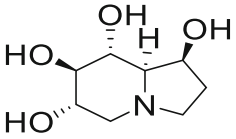
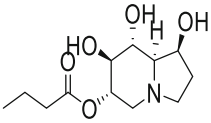
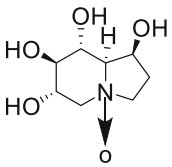
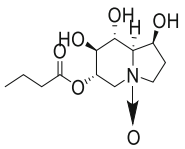
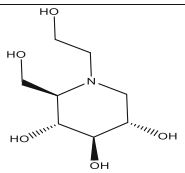
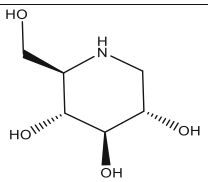


Fig. 13 Probable fragmentation pattern of celgosivir and n-oxide impurity

Product Name	Structure	Chemical Name	Empirical Formula
Celgosivir		(1S,6S,7R,8R,8aR)-1,2,3,5,6,7,8,8a-octahydroindolizine-1,6,7,8-tetrol	$C_8H_{15}NO_4$
Castanospermin		(2R,3R,4R,5S)-1-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-2-(hydroxymethyl)piperidine-3,4,5-triol	$C_{12}H_{21}NO_5$
Celgosivir n-Oxide imp		---	$C_8H_{15}NO_5$
Castanospermin n-Oxide imp		---	$C_{12}H_{21}NO_6$
Miglitol		(2R,3R,4R,5S)-1-(2-hydroxyethyl)-2-(hydroxymethyl)piperidine-3,4,5-triol	$C_8H_{17}NO_5$
DNJ		(2R,3R,4R,5S)-2-(hydroxymethyl)piperidine-3,4,5-triol	$C_6H_{13}NO_4$

DNJ:1-deoxynojirimycine

Fig. 14 Selected Aza sugars product names, structures, chemical names, and empirical formulae

peaks were at 276 and 206 m/z for n-oxides of celgosivir and castanospermin respectively. At higher collision energies, the stable molecular ion peaks were at 206 and 188 m/z for n-oxides of celgosivir and castanospermin respectively. The hydroxyl group loss stated that 206 and 188 m/z for celgosivir and castanospermin, respectively, was observed. Further fragmentation gave continuous loss of hydroxyl group by using higher collision energies (Figs. 8, 9, 10, and 11). The fragmentation pattern difference observed was 16 mass number such as APIs and its n-oxide impurities (Table 1, Additional file 1). HR-MS data supported the fragmentation profile of n-oxides of celgosivir and castanospermin and characterized as n-oxides of celgosivir and castanospermin (Figs. 12, 13, and 14; Table 2).

Method validation for castanospermin and celgosivir by ion chromatography

Specificity

Chromatography obtained with mixer of castanospermin and celgosivir shows no interference with other

Table 2 HR-MS pseudo fragmentation profile for formulae confirm of the n-oxide impurity of castanospermin and celgosivir

Name of product	Fragmentation	Formulae
Castanospermin	190.1070 $[M + H]^+$	$C_8H_{16}NO_4$
	172.0965	$C_8H_{14}NO_3$
	154.0872	$C_8H_{12}NO_2$
	136.0753	$C_8H_{10}NO$
	112.0743	$C_6H_{10}NO$
n-Oxide impurity of CAS (unknown impurity)	206.1027 $[M + H]^+$	$C_8H_{16}NO_5$
	172.0968	$C_8H_{14}NO_3$
	154.0857	$C_8H_{12}NO_2$
Celgosivir	260.1486$[M + H]^+$	$C_{12}H_{22}NO_5$
	172.0972	$C_8H_{14}NO_3$
	154.0834	$C_8H_{12}NO_2$
	242.1272	$C_{12}H_{20}NO_4$
Oxide impurity of CEL (unknown impurity)	276.1448 $[M + H]^+$	$C_{12}H_{22}NO_6$
	206.1008	$C_8H_{16}NO_5$
	158.0816	$C_7H_{12}NO_3$

Aza sugars such as 1-deocynojirimycine, miglitol, and their degradants. During the degradation study, the n-oxide impurities of castanospermin and celgosivir were formed with 3% hydrogen peroxide (Table 3) and no interference with other cations. The n-oxide impurities of castanospermin and celgosivir were identified by isolation at the column end and characterized by high-resolution mass spectrometry and LC-MS/MS. Degradation study of castanospermin and celgosivir were performed by acid hydrolysis (0.5N HCl), base hydrolysis (0.5% Tri ethyl amine and 0.5N NaOH), oxidation (3% H202), photo degradation at 1.2 million lux hours, visible light, 200 W/m^2 UV light, and thermal degradation at 105 °C. The degradation peaks have been identified and characterized as n-oxides of castanospermin and celgosivir by high-resolution mass spectrometry and LC-MS/MS. The peak purity was evaluated by high-resolution mass spectrometry and LC-MS/MS. The mass balance is demonstrated in Table 3.

Precision

The precision data obtained for the evaluated method is demonstrated in Table 4. The precision at 50, 100, and 150% were evaluated for castanospermin and celgosivir in a mixer solution. The %RSD at ($n = 6$) all levels were less than the 2.0% assuming acceptable precision.

Accuracy

Accuracy was performed by means of recovery studies using the developed method. The percentage of recoveries after spiking with additional mixer of castanospermin and celgosivir was at all levels such as 50, 100, and 150% in the range of 98–102%, and the results are listed in Table 4.

Linearity

A linear relationship was presented between the concentration and peak area. The linearity concentration for the mixer of castanospermin and celgosivir was taken to seven levels such as 25, 50, 75, 100, 125, 150, and 200%. The correlation coefficient value (r) and regression coefficient value (r) obtained for both was more than 0.999, which explains the linearity of the method, and the results are listed in Table 4, (Plot 1 and Plot 2).

Table 3 Summary of forced degradation results

Stress condition	Duration	Assay of after forced degradation castanospermin (%*w/w*)	Assay of after forced degradation of celgosivir (%*w/w*)	Content of major degradant (%*w/w*)	Remarks
Acid hydrolysis	10 days	100	100	–	No degradation products formed for both
Base hydrolysis	0 h	.0	100	100 of CAS formed from CIL after 0 h	No degradation products formed for CAS
Oxidation	6 h	79.2	90.0	20.8% n-oxide of CAS, 10% of n-oxide CIL	n-oxides both sugars
Thermal (105 °C)	7 days	100	100	–	No degradation products formed for both
UV light	200 W/m^2	100	100	–	No degradation products formed for both
Visible light	1.2 million lux hours	100	100	–	No degradation products formed for both

Table 4 Summary of validation results

Parameter	Castanospermin	Celgosivir
Correlation coefficient (*r*)	1.000	0.999
Intercept (*c*)	− 0.01	− 0.01
Slope (m)	0.1	0.03
Method precision (%RSD)	1.1	0.7
Intermediate precision (%RSD)	3.2	3.1
Precision at 50%	1.2	1.7
Precision at 150%	0.4	1.9
Accuracy at 50% (%recovery)	100.1	95.4
Accuracy at 100% (%recovery)	99.7	99.7
Accuracy at 150% (%recovery)	97.0	82.3
Precision at flow 0.6 mL/min (%RSD)	0.5	0.4
Precision at Flow 0.8 mL/min (%RSD)	1.1	0.7
low buffer strength (%RSD)	0.5	1.5
High buffer strength (%RSD)	2.4	0.5
Solution stability and mobile phase difference	− 1.0	− 8.5
With respect to initial (%recovery variation)		

Robustness

Robustness was performed by changing flow, i.e., from 10% to lower to actual flow and 10% higher flow from actual flow, and buffer concentration flow, i.e., the study has been performed from 10% to lower concentration and 10% higher concentration from actual concentration. The evaluated parameters were performed for the precision, %RSD were calculated and the results are listed in Table 4.

Ruggedness

The %RSD values for intermediate precision and repeatability reported were found to be less than 2% which shows ruggedness of the ion chromatography method. The results of ruggedness parameters are listed in Table 4.

Solution and mobile phase stability

Solution stability was performed with same mixer of castanospermin and celgosivir in active pharmaceutical ingredient solution at 24 and 48 h. The mobile phase was observed as stable in fresh mixer (for the assay of castanospermin and celgosivir in active pharmaceutical ingredients) at 24 and 48 h. The % variations of assay for both are less than − 8.5%.

Conclusions

The present research provides the ion chromatographic method for the assay determination of the castanospermin and celgosivir as active pharmaceutical ingredients. Identification of the other Aza sugars such as 1-deoxynojirimycin and miglitol in the same method with less amount of the sample quantity was determined. The method was validated as per ICH Q2 (R1); the proposed ion chromatography method is found to be simple, sensitive, accurate, precise, specific and green chemistry type of analysis for diluent and mobile phase preparation; it can be used for intended purposes in drug substance and drug product. The n-oxide degradation impurities of castanospermin and celgosivir under peroxide degradation were identified and characterized by using the high-resolution mass spectrometry and the LC-MS/MS. These novel techniques will help to improve the quality of the drugs because the most of Aza sugars are using in diabetic research and dengue research.

Additional file

Additional file 1: Fragmentation pattern differences. (DOCX 761 kb)

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Authors' contributions

PM, JMB, KB, and DRD conceived and designed the experiments. NR and BbD performed the experiments and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no that they have no competing interests.

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