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Research Article

CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS IDENTIFICATION OF ASPARTAME, SACCHARIN AND SUCRALOSE IN TRADITIONAL INDIAN SWEETS SAMPLES BY HPTLC AND CHARACTERISATION BY HPTLC-MS

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ABSTRACT

The present paper reports a HPTLC-MS method for the simultaneous detection and identification of the artificial sweeteners aspartame, saccharin and sucralose in two Indian sweet foodstuffs viz. ‘kesar pedha’ and ‘yellow mawa barfi’. HPTLC method for identification has been developed using Silica gel 60 F254 HPTLC plates, with optimised mobile phase i.e. chloroform: methanol: water: acetic acid 64:50:10:0.1 v/v/v/v in Automatic Development Chamber (ADC2) under controlled humidity of about 33% using $MgCl_2$ saturated salt solution. HPTLC in combination with MS was used for routine analysis of aspartame, saccharin and sucralose which are added as artificial sweetner to traditional Indian sweet products. TLC-MS analysis confirmed the presence of Aspartame (294 g/mol), Sucralose (397.64 g/mol) and saccharin (182 g/mol) in the test samples of yellow mawa barfi and kesar pedha.

Keywords: Food additives, aspartame, saccharin, sucralose and HPTLC-MS.

INTRODUCTION

Artificial sweeteners are used to impart a sweet taste to foodstuffs. These sweeteners are helpful in controlling body weight and insulin levels as they provide no or little calories. Sweeteners may be used separately or in combination with other sweeteners. Because different sweeteners can elicit different sweet taste quality, 2 or 3 different artificial sweeteners are added in one type of food simultaneously to produce a good sweet taste. Since the sweeteners may be used illegally or used more than the legal content limits, a simple, sensitive, and reliable simultaneous determination of these sweeteners is required [1].

Various methods based on different principles have been reported for the simultaneous determination of the sweeteners[2,3,4].

The use of artificial sweeteners like saccharin, acesulfame-k, aspartame and sucralose has been permitted in halwa, khoya barfi, rasogulla, gulabjamun and other milk products[5,6].

Aspartame is a dipeptide methyl ester of L-aspartyl-L-phenylalanine. Aspartame is about 200 times sweeter than sucrose. It is a white crystalline, odorless intensively sweet powder which has the molecular formula $C_{14}H_{18}N_2O_5$ along with the molar mass 294.31 g/mol[7].

The FSSAI manual of ‘Food additives 2016 recommends the use of traditional thin layer chromatography for the qualitative determination of aspartame for dry beverage powders[8].

Saccharin, a petroleum derivative is a white crystalline artificial sweetener that is about 300 to 500 times sweeter than sucrose. Saccharin is not readily soluble in water, but its commercially available sodium salt is freely soluble in water. Saccharin is highly stable under food processing and storage conditions. It can withstand heating, baking and acid media. At very low pH however, saccharin is hydrolysed into 2-sulfobenzoic and 2-sulfamoylbenzoic acids.

It is one of the most studied food ingredients and the foundation of many low-calorie and

sugar-free products around the world [9, 10].

The FSSAI manual of 'Food additives 2016 recommends the use of traditional thin layer chromatography for the qualitative determination of saccharin[8].

Sucralose is a chlorinated carbohydrate non-nutritive sweetener of food and beverage products. It is thermally stable and about 600 times sweeter than sucrose and can be used during cooking and baking. Sucralose is readily soluble in ethanol, methanol and water and this solubility profile contributes to its versatility in both fat- and water-based food and beverage applications including alcoholic drinks[11,12,13].

Among the wide choice of chromatographic techniques, HPTLC is a simple, fast and accurate technique for use, making it advantageous over others for quick assessment of a number of samples simultaneously[14]. Rapid and reliable High-Performance Thin Layer Chromatographic method has been developed to detect and identify the artificial sweetener aspartame, saccharin and sucralose from the sample extracts. As complex extraction method causes delays in analysis and may lead to improper results, a simple and rapid extraction method has been developed.

The combination of TLC and MS provides a means to characterize the chemical compounds after their chromatographic development. Mass spectrometry (MS) is one of the most practical techniques for characterizing chemical and biochemical compounds. By measuring the mass-to-charge (m/z) ratios of ions, it allows elucidating the chemical structures of molecules in terms of the species formed through fragmentation in the ion source or mass analyser[15].

A mass spectrometric detector can be off-line coupled with the thin-layer chromatographic plate by means of the TLC–MS interface. Thus, HPTLC has an additional analytical dimension which enhances its performance, thus becoming an even more flexible and better performing separation and identification tool than before.

It possesses the necessary flexibility for combining various complementary detection modes such as mass spectrometry (MS), which has proven to be useful to identify such additives in food stuffs [16,17].

In the present research paper HPTLC method has been developed for the simultaneous determination of aspartame, saccharin and sucralose in traditional Indian sweet preparations like yellow mawa barfi and kesar pedha and HPTLC-MS analysis was carried out to confirm the presence of these sweeteners in those sweet products used for the present study.

MATERIAL AND METHODS

CHEMICALS AND REAGENTS

Methanol, acetic acid, water, chloroform, zinc sulphate, potassium ferrocyanide, isopropanol, ninhydrin, diphenylamine, acetone, orthophosphoric acid (Merck) were used. All the organic solvents used in the study were of analytical grade.

INSTRUMENTATION

High performance thin layer chromatography system (CAMAG, Muttenz, Switzerland) consisted of Linomat V, TLC Visualizer I, CAMAG Automatic Development Chamber (ADC 2) and TLC Scanner 4 attached to a PC running visionCATS software (version 2.5) were used in analysis.

Combination Standard solution preparation

Aspartame, saccharin and sucralose standard were weighed to 1mg/mL each and volume was made up to 10 mL with water. Combination working standard of 0.1mg/mL was used for analysis.

Sample preparation

A. yellow mawa barfi, kesar pedha samples were collected from different localities of Mulund East, Mumbai. Each respective sample was weighed and macerated to 1 g with addition of 10.5 mL distilled water and 2.5mL petroleum ether and 1mL Carrez solution I and II each. This solution was cold sonicated for 10 minutes and centrifuged at 2500 rpm for 15 minutes. After centrifugation second last layer i.e. aqueous layer was collected and directly used for application.

Sample clarification solutions

A. Carrez Solution I (Clarification solution): Potassium ferrocyanide weighing 3.6 g was dissolved in 100 mL water.
B. Carrez solution II (Clarification solution): Zinc sulphate weighing 7.2 g was dissolved in 100 mL water.

Post derivatization reagent preparation:

A. **Ninhydrin reagent**
   Ninhydrin (2,2-dihydroxyindene-1,3-dione) weighing 0.6g was placed in a glass bottle and dissolved in 190 mL of isopropanol and 10mL of concentrated acetic acid.

B. **Aniline diphenylamine orthophosphoric acid**
   Weigh 1gm diphenylamine and add 1mL aniline in 80 mL methanol with addition of 10mL orthophosphoric acid. Make up to 100mL with methanol.

CHROMATOGRAPHIC ANALYSIS

TLC plate (20 × 10 cm; 0.2 mm thick coated with silica gel 60 F_{254}) having 15 tracks of samples and standards were used under following conditions: band width 8 mm, track distance 11.4 mm, sample application with 8 mm from lower edge and 20 mm from left and right edge of the plate sprayed using Linomat V with 100 µl syringe. Applied plates were developed to a distance of 70mm in Automatic Development Chamber (ADC 2) with 33% humidity control by using $MgCl_2$ saturated salt solution. The mobile phase optimized was chloroform: methanol: water: acetic acid 64:50:10:0.1 v/v/v/v. Saturation pad was used and saturation was done for 20 minutes. Two such plates were developed with above mentioned parameters as sucralose and aspartame detection need different derivatizing reagents. The developed plates were derivatized with Ninhydrin reagent for aspartame and with aniline diphenylamine orthophosphoric acid (ADP) for sucralose and saccharin was detected in UV. The images were documented using CAMAG visualizer I at white light, 254nm and at 366nm after derivatization with ADP for aspartame, saccharin and sucralose respectively. The plates were scanned at 520 nm, 230 nm and 366nm using CAMAG Scanner 4 for aspartame, saccharin and sucralose respectively. Scanning conditions were optimized as: slit dimension, 6.00 mm × 0.45 mm (micro); scanning speed, 20 mm/s; and data resolution, 25 µm per step. Scanning data was automatically generated by the visionCATS software version 2.5.

Post chromatographic derivatization for aspartame and sucralose

Two different plates were prepared for two respective derivatizing reagents. The right part of the plate was tightly wrapped with a double layer of aluminum foil. Then the left part was manually immersed in Ninhydrin reagent and aniline diphenylamine orthophosphoric acid reagent for aspartame and sucralose respectively. After derivatization the positions of the fractions were determined and marked with pencil as per the position/Rf with derivatized part and respective plates were subjected to TLC-MS analysis.

HPTLC-MS INSTRUMENTATION

HPTLC-MS analysis was carried out by using CAMAG TLC-MS interface II with oval elution head. The chromatogram zones were eluted from the HPTLC plate with methanol with the flow speed appropriate for the LC-MS system. Laser crosshair was used to exactly position the zones of interest under the extraction piston of the interface. The TLC-MS interface was operated in a semi-automatic mode. Moving a lever starts the solvent flow through the layer and extracts the zone. After each sample extraction cleaning process was run to avoid carryover. After elution the eluate was directly transferred to the mass spectrometer for further offline analysis. The TLC-MS conditions were set after optimization which are as follows

Mobile phase: Water: Methanol (40:60) v/v

Flow rate: 0.3mL per minute ,

Mass Spectrophotometer- (Shimadzu Single quadrupole 2020), LC pump with low pressure gradient in ESI-APCI mode. Analysis was carried out in negative polarity with 0 threshold value. m/z range was from 50 to 500. Software used was LabSolutions LCMS.

RESULTS AND DISCUSSION

Method development

The sample preparation procedure was standardized for the isolation of sweeteners aspartame, saccharin and sucralose in kesar pedha and yellow mawa barfi samples. The method involved ultrasonification, Carrez clarification (protein precipitation) and filtration. The proposed HPTLC method was optimized for separation and resolution for sample extraction, composition of mobile phase, nature of stationary phase, time of saturation, derivatizing reagent. Standardized conditions for HPTLC analysis were as follows: optimized mobile phase i.e. chloroform: methanol: water: acetic acid 64:50:10:0.1 v/v/v/v, stationary phase i.e. Si $60F_{254,}$ time of saturation i.e. 20 minutes. Aspartame, saccharin and sucralose were observed at Rf value of 0.58, 0.66 and 0.74 respectively. Scanning was carried out at 520nm, 230nm and 366nm for aspartame, saccharin and sucralose respectively and images were recorded (Figure 1, 2, & 3)

TLC-MS results

TLC-MS analysis recorded mass spectra of standard aspartame as 293 g/mol. As analysis was performed in negative ionization, mass spectra was found matching with its reported value of 294.31 g/mol. In barfi sample aspartame was recorded at mass 293 g/mol (Figue 4, 5).

TLC-MS analysis recorded mass spectra of standard sucralose as 397 g/mol. As analysis was performed in negative ionization, mass spectra was found matching with its reported value of 397 g/mol In barfi and kesar pedha sample sucralose was recorded at mass 397 g/mol (Figure 6, 7 & 8).

TLC-MS analysis recorded mass spectra of standard saccharin as 182 g/mol. As analysis was performed in negative ionization, mass spectra was found matching with its reported value of 182 g/mol. In barfi and kesar pedha sample saccharin was recorded at mass 182 g/mol

(Figure 9, 10 & 11).

CONCLUSIONS

The present paper reports a simple HPTLC method for the routine analysis of three sweeteners viz. aspartame, saccharin and sucralose in two Indian sweet samples. The same method can be adopted by regulatory authorities for accurate determination of aspartame, saccharin and sucralose in yellow mawa barfi and kesar pedha samples. This method is relatively simple, fast and accurate for the determination of these sweeteners in above mentioned samples.

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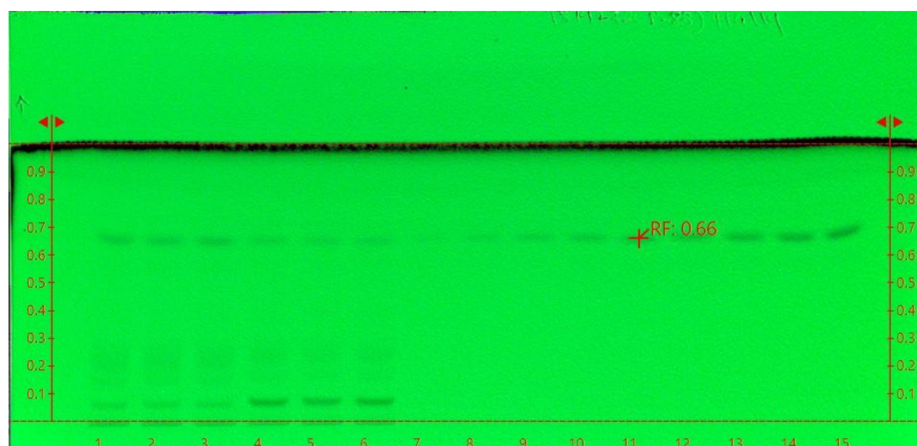

**Fig. 1: Image at UV 254nm for Saccharin Std. in barfi and pedha Sample.
Track 1-3: barfi, Track 4-6: Pedha, Track 7-15: Saccharin standard**

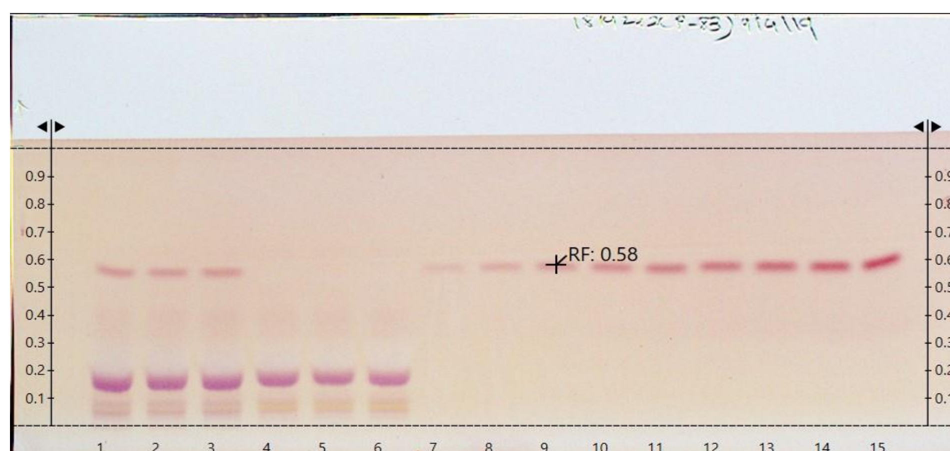

**Fig. 2: Image at visible light post derivatisation under white light for Aspartame Std. in barfi Sample.
Track 1-3: barfi, Track 4-6: pedha, Track 7-15: Aspartame standard**

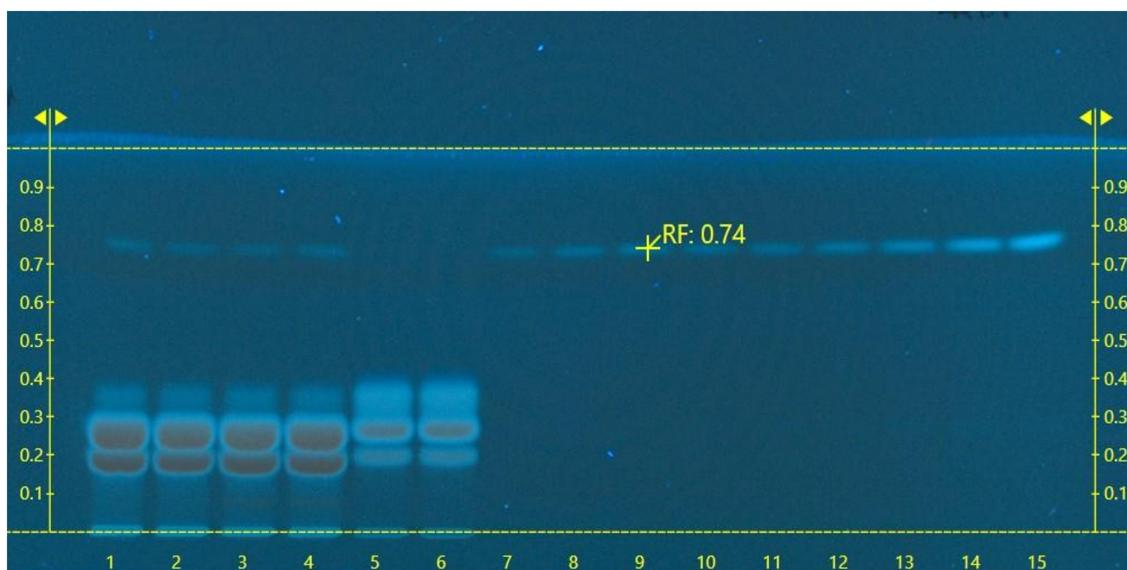

Fig. 3: Image at 366nm post derivatisation for Sucralose Std. in barfi and pedha Sample. Track 1-2: barfi, Track 3-4: pedha, Track 5-6: Lassi, Track 7-15: Sucralose standard

Retention Time: 55.39
Mode: -ve
m/z: 293
Mass Spectra:

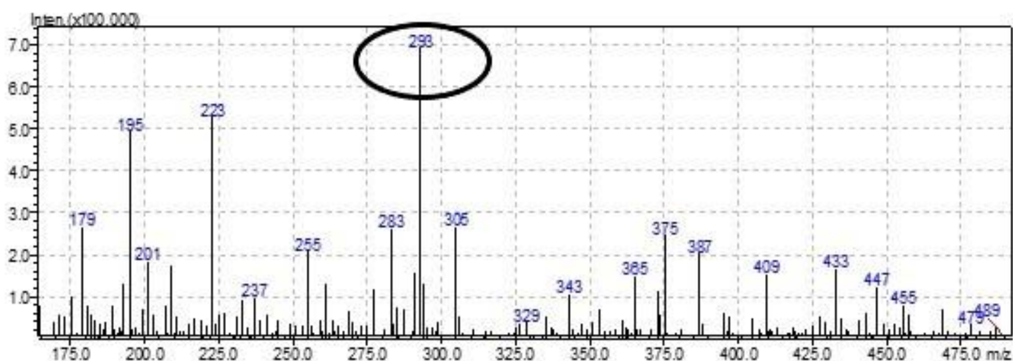

Fig. 4: Mass spectra for standard aspartame

Retention Time: 49.39
Mode: -ve
m/z: 293
Mass Spectra:

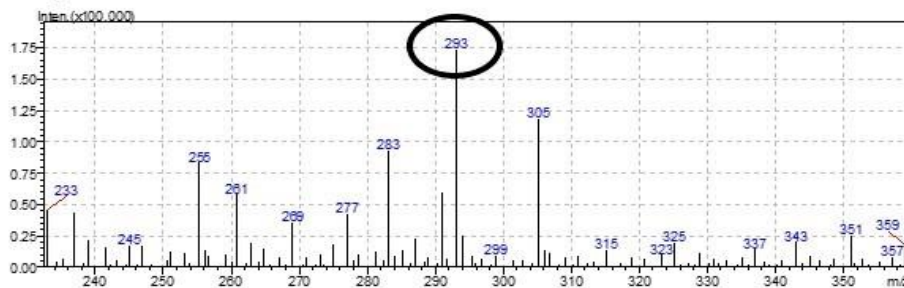

Fig. 5: Mass spectra for aspartame in yellow mawa barfi sample

Retention Time: 89.46

Mode: -ve

m/z: 397

Mass Spectra:

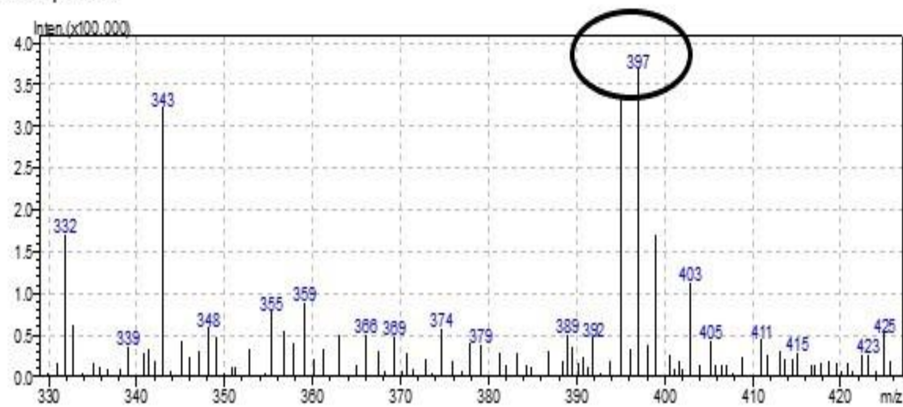

Fig. 6: Mass spectra for sucralose standard

Retention Time: 84.51

Mode: -ve

m/z: 397

Mass Spectra:

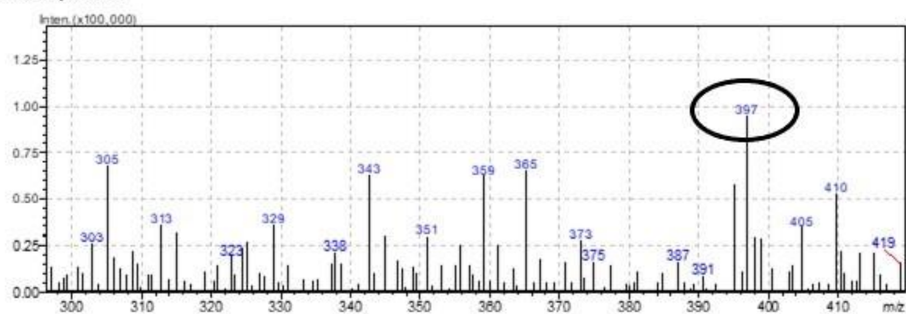

Fig. 7: Mass spectra for sucralose standard in yellow mawa barfi sample

Retention Time: 87.37

Mode: -ve

m/z: 397

Mass Spectra:

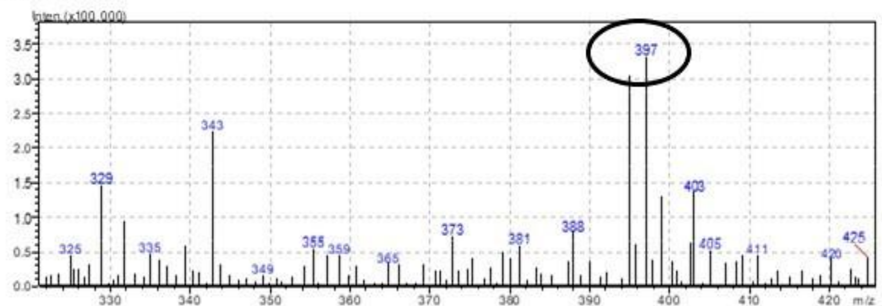

Fig. 8: Mass spectra for sucralose standard in kesar pedha sample

14. Retention Time: 98.22
Mode: -ve
m/z: 182
Mass Spectra:

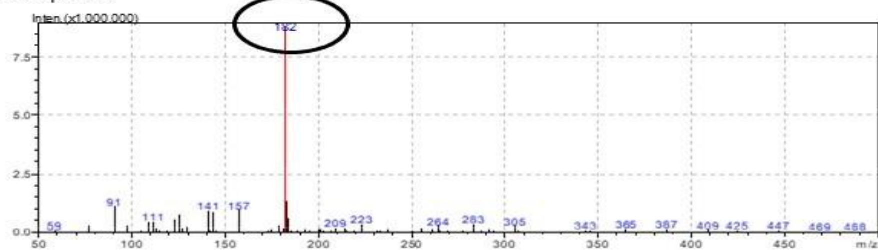

Fig. 9: Mass spectra for saccharin standard

Retention Time: 9.82
Mode: -ve
m/z: 182
Mass Spectra:

Fig. 10: Mass spectra for saccharin standard in yellow barfi sample

Retention time: 22.67
Mode: -ve
m/z: 182
Mass Spectra:

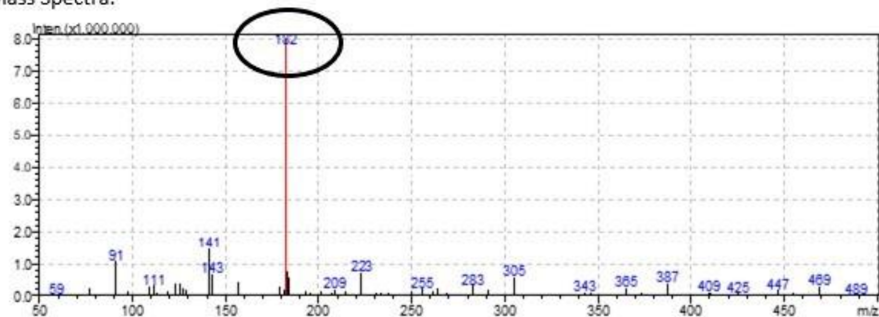

Fig. 11: Mass spectra for saccharin standard in kesar pedha sample

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