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Evaluation and method validation of sodium cyclamate in watermelon by LC-MS/MS

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Abstract
Watermelon is a common fruit used by most of the people in summer seasons. Sodium cyclamate is ideal for use by people suffering from diabetes. Literature shows that very less work has been done on estimation of sodium cyclamate in watermelon using LC-MS/MS. In the present study an attempt was made to develop a method for estimation of Sodium cyclamate in watermelon and is validated for its accuracy, precision, linearity, range and other validation parameters. The results showed that for the linearity the correlation coefficient was found to be 0.999, in the range of 10-1000ng/mL. The limit of detection is 1ng/mL, the limit of quantification is 3ng/mL. The RSD for six replicate injections of sodium cyclamate at 10ng/mL level were established and % RSD was found to be 3.7. Recovery was found to be 88.5% for sodium cyclamate in watermelon @ 200ng/mL.

Keywords: sodium cyclamate, watermelon, LC-MS/MS, artificial sweeteners

1. Introduction
Artificial sweeteners are used as a sugar substitute in food and beverage products such as cereal bars and soft drinks. Their use is becoming more popular as consumers are increasingly concerned about obesity and dental decay caused from consuming natural sugars. In addition, artificial sweeteners are ideal for use by people suffering from diabetes. Acesulfame potassium, saccharin, aspartame, and sucralose are all commonly used artificial sweeteners that have been approved for use by the regulatory authorities. Sodium cyclamate has been banned in the United States, although it is approved for use in more than 50 countries worldwide, including the UK.
Cyclamate is a non-caloric sweetener discovered in 1937. It has been used widely in low-calorie foods and beverages. When cyclamate is combined with other low-calorie sweeteners, they enhance each other so that the combinations are sweeter than the sum of the individual sweeteners. So, cyclamate has been widely used as a tabletop sweetener, particularly in combination with saccharin. For the determination of cyclamate, derivative gas chromatography (GC) and high-performance liquid chromatography (HPLC) are most widely used [2, 3, 5]. However these methods are often complicated and tedious.
However, some findings in animals suggested that cyclamate might increase the risk of bladder cancer in humans, the U.S. Food and Drug Administration (FDA) banned the use of cyclamate in 1969. Although more recent animal studies have failed to demonstrate that cyclamate is a carcinogen or a co-carcinogen, other issues must be resolved before cyclamate can be approved for commercial use as a food additive in the United States [1]. Hence in recent days proper estimation of sodium cyclamate in watermelon is gained more importance. In the present study a precise, accurate method of estimation of sodium cyclamate in watermelon is attempted using LC-MS/MS.

2. Experimental Details
2.1 Reagents and chemicals
All Chemicals/Reagents were of LC - MS grade and used without any further purification. Stock standard solution of Sodium Cyclamate was prepared (100 µg/mL) by dissolving 2.5 mg of the pure analytical standard in 25 mL of water. A series of standard solutions were then gained by the appropriate dilutions of the above mentioned stock solutions with 0.1% formic acid to get 10, 50, 100, 200, 500 and 1000ng/mL. All the stock solutions and working solutions were stored in a refrigerator and brought to room temperature before use.

2.2 Sample preparation and extraction
As cyclamate has good solubility in water, samples were extracted by water, the whole fruit of watermelon (only the pulp portion) is mashed to get juice, the juice was then centrifuged at 12000rpm for 10min and the resulting juice was acidified with 0.1% formic acid. The supernatants were filtered through a 0.45µ nylon membrane. Then a 10µl aliquot of the sample solution was injected into LC-MS/MS system connected with Atlantis T3 column using gradient method of 0.1% formic acid in water and 0.1% formic acid in acetonitrile as a mobile phase. Because the sensitivity of the method was high, the concentrations of cyclamate in final sample solution should be controlled in the linear range. Specially, when the concentrations of cyclamate in samples were high, the sample solution must be dilute.

3. Results and Discussion
3.1 Specificity
3.1.1 Specificity of the method
To check the specificity of the method the Sodium Cyclamate is injected at 10ng/ml in six replicates separately and sample blank solution and reported for no interference of sodium cyclamate in blank.

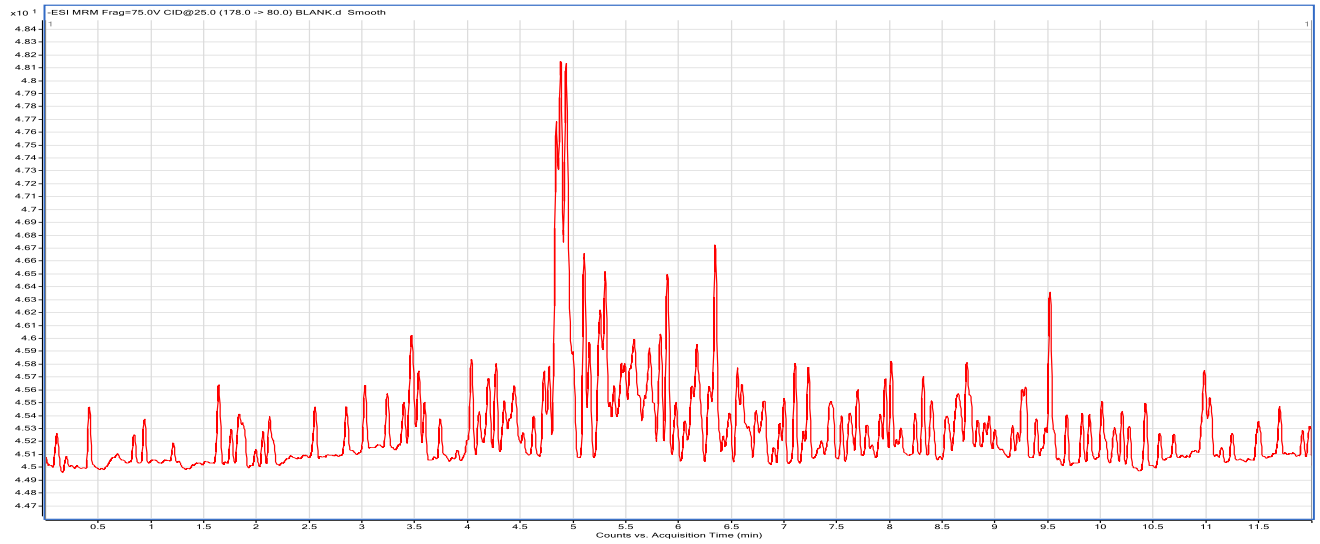

Fig 1: Blank Chromatogram of Sodium Cyclamate

3.2 Linearity

Solutions of lower concentrations of sodium cyclamate standard were prepared as solvent standard and matrix match standard and each concentration was injected on the same day. The data generated was analyzed by linear regression analysis to calculate the slope, intercept and the correlation coefficient. Linearity graphs are plotted. The method follows linear range over 10ng/ml to 1000ng/ml for sodium cyclamate in watermelon respectively with a correlation coefficient greater than 0.99.

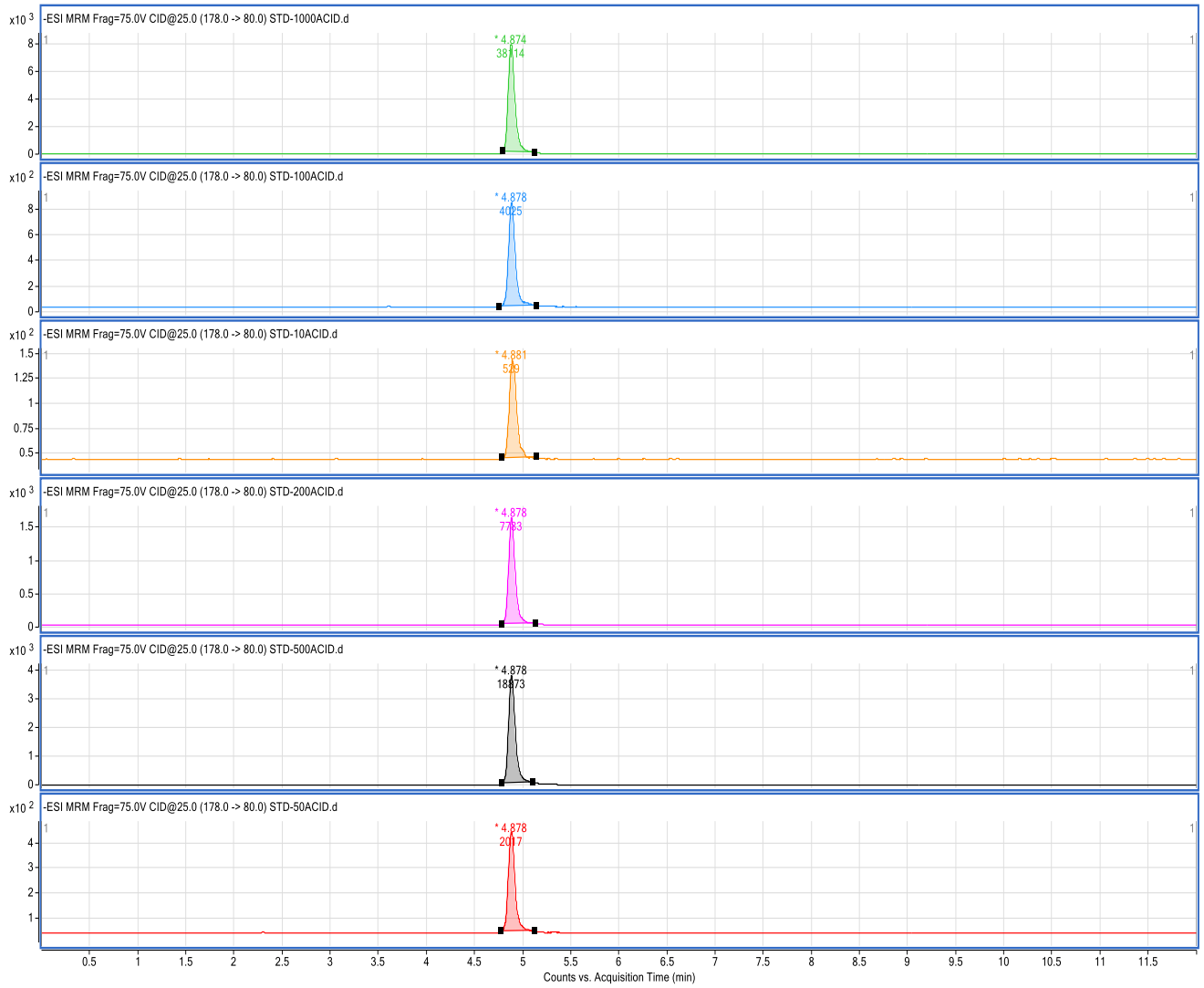

Fig 2: Linearity of Sodium Cyclamate

3.3 Precision

Precision is carried out during experiment by injecting the six no. of replicate injections of the sodium cyclamate STD at 10ng/ml. The RSD for six replicate injections of Sodium Cyclamate at 10ng/ml level were established and % RSD was found to be 3.7.

Fig 3: Precision of Sodium Cyclamate

3.4 Limit of Detection (LOD) & Limit of Quantification (LOQ)

For prediction of LOD and LOQ, series of standard solutions containing lower concentrations of sodium cyclamate is prepared and analyzed. From these concentrations derive the concentration for LOD and LOQ by considering the S/N ratio. In addition cyclamate resulted in a quite low LOD and LOQ. According to the USA FDA criteria [6], the analyte response at the LOQ should be at least five times the response of blank baseline. With acceptable repeatability, recovery, the LOQ of cyclamate was 3 ng/mL. The analyte response at the limit of detection (LOD) should be reliably differentiated from background noise. The LOD of cyclamate was 1 ng/mL.

3.5 Accuracy

The mean recovery of the target compound was investigated by adding known concentrations to the sample at 200ng/mL. Recovery was found to be 88.5% for sodium cyclamate in watermelon @ 200ng/mL. Which is within the prescribed limits i.e., from 70% to 120%. [4].

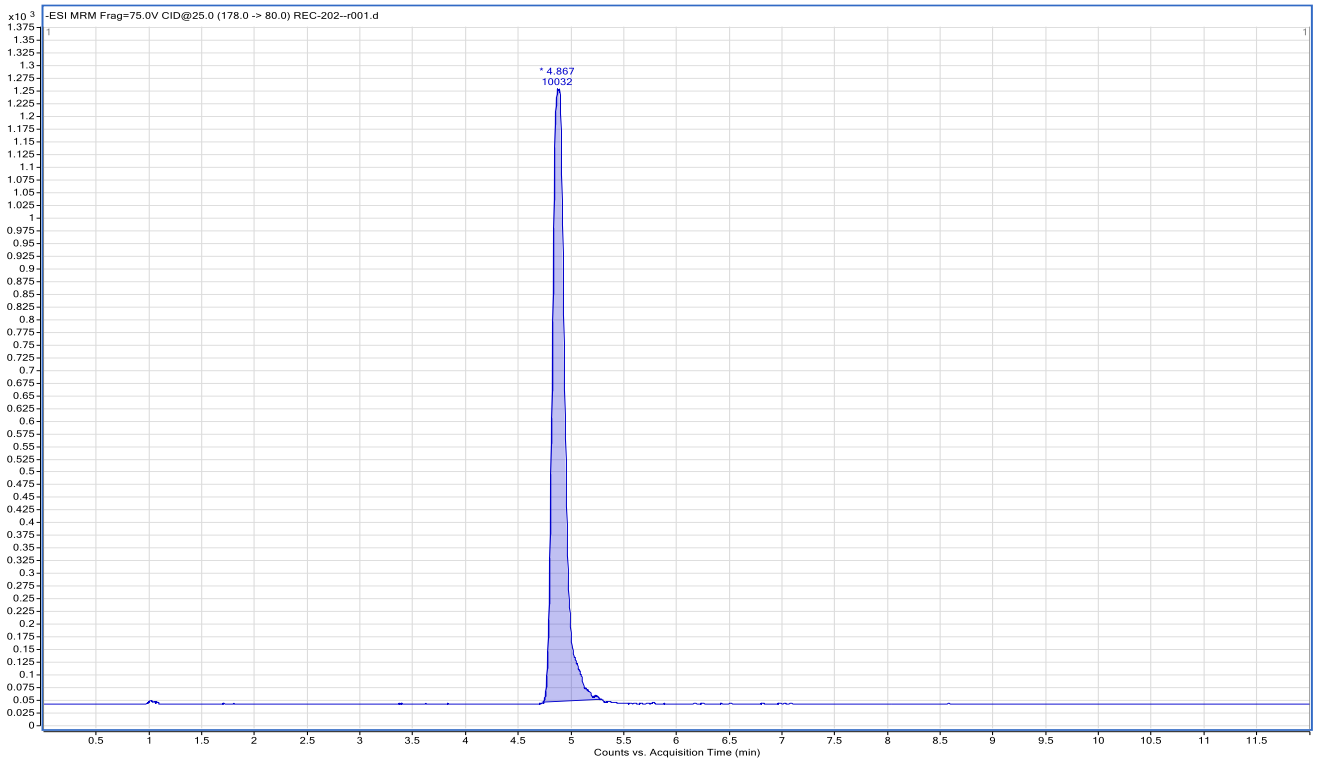

Fig 4: Recovery Chromatogram for Sodium Cyclamate @ 200ng/mL

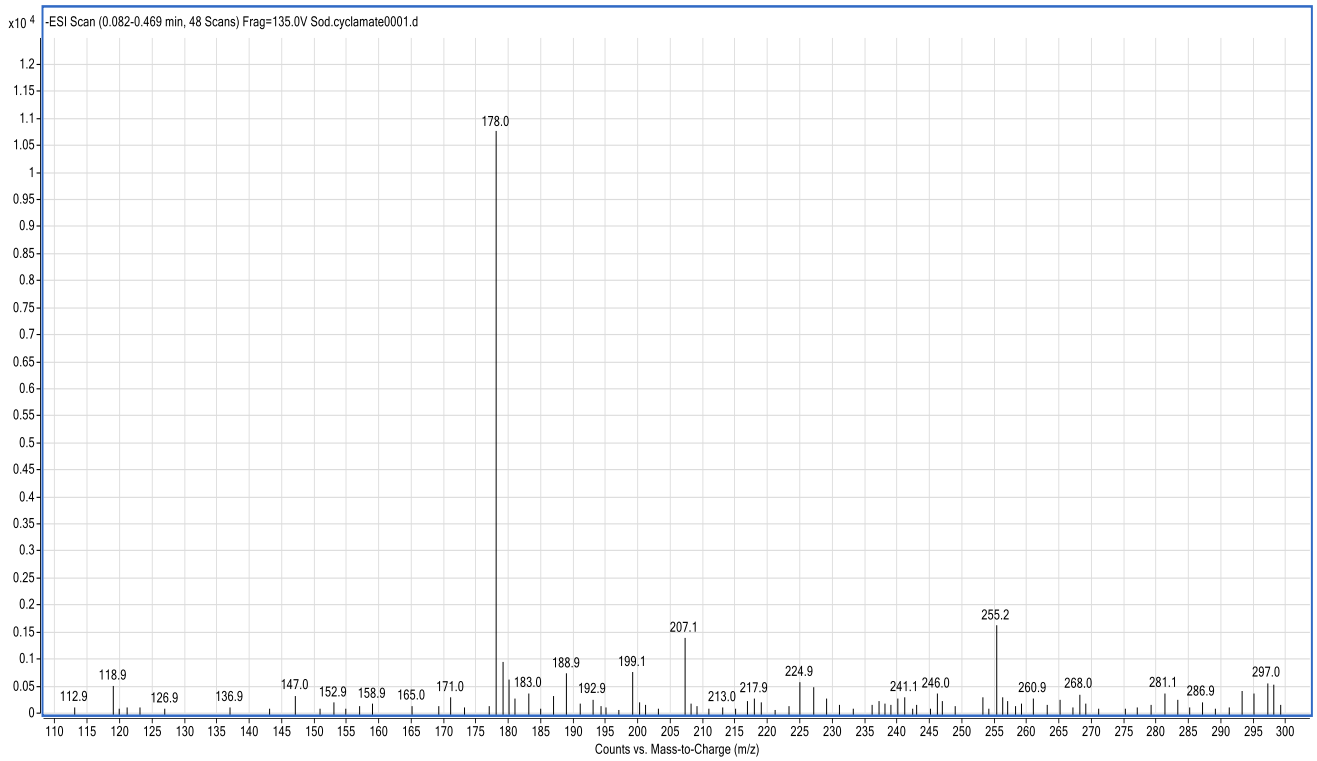

Fig 5: MS Spectra of Sodium Cyclamate

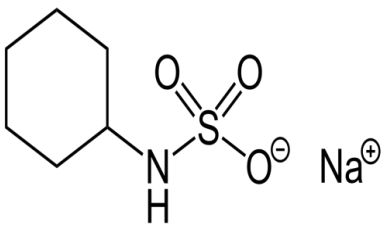

Fig 6: Structure of Sodium Cyclamate

Table 1: Validation Summary

S. No	Validation Parameters	Observations	Acceptance Criteria
1	Specificity	Method found specific for sodium cyclamate in watermelon. No interference was observed by analyte or compound at the retention time of the same analyte in the blank	The sodium cyclamate peaks in watermelon should be well resolved
2	Linearity	The method is linear for sodium cyclamate in watermelon over concentration range of 10ng/mL, 50ng/mL, 100ng/mL, 200ng/mL, 500ng/mL and 1000ng/mL with correlation coefficient greater than 0.99	Correlation coefficient greater than or equal to 0.99
3	Limit of Detection (LOD)	1ng/mL of RS concentration for sodium cyclamate in watermelon	Signal to Noise Ratio should be above 3 and % RSD for six injections at this concentration should be less than 20%
4	Limit of Quantification (LOQ)	3ng/mL of RS concentration for sodium cyclamate in watermelon	Signal to Noise Ratio should be above 103 and % RSD for six injections at this concentration should be less than 20%
5	Precision	%RSD is within desired limits for 10ng/mL,	% RSD should not be more than 20.0
6	Accuracy	Recovery is within the prescribed limit for Sodium cyclamate in watermelon @200ng/mL.	Mean recovery should lie within 70% to 120%

4. Conclusion

The validation study showed that good separation was achieved on a Atlantis T3 column with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phase with gradient elution. The quantification of target compound was completed using a selected ion recording (SIR) at m/z 178 obtained from ESI-mode. The results show that LC-MS/MS is an effective method for the estimation of Sodium Cyclamate in watermelon at ng/mL level.

5. References

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