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# Development of an analytical method for estimation of neotame in cake and ice cream

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ABSTRACT

A solid phase extraction method using $C_{18}$ cartridge was standardized for the isolation of neotame from cake and ice cream. High performance liquid chromatography (HPLC) method was developed and validated for estimation of neotame in cake and ice cream. The developed HPLC method was simple, precise, accurate, reproducible and sensitive. Mobile phase consisted of 0.09% TFA, acetonitrile:water (60:40) and the flow rate was maintained at 0.6 ml/min. HPLC separation of neotame was carried out on a reverse phase $C_{18}$ column with photo diode array detector at 210 nm. The recovery of neotame from cake and ice cream by the developed method ranged from 96.08 to 98.62%. At baking temperature (180 °C/20 min) 87.29% of the neotame remained intact, however the amount of neotame decreased significantly from 87.20 to 62.40% during storage (20 days at 25 °C). Pasteurization (68 °C/30 min) resulted in no loss of neotame in ice cream mix; however the amount of neotame decreased significantly from 99.42 to 89.93% during storage (90 days at −18 °C). The developed HPLC method can be successfully used for the routine determination of neotame in cake and ice cream formulations.



## 1. Introduction

Neotame is a non-calorie sweetener and has a sweetness factor approximately 7,000 to 13,000 times greater than sucrose and approximately 30–60 times greater than aspartame (EFSA, 2007). As a derivative of aspartame it has inherent qualities of aspartame i.e. clean sugar-like taste, with no undesirable metallic or bitter taste, but used at lower level (Nofre & Tinti, 2000). Moreover, it has additional feature over aspartame, as higher stability in the neutral pH range which allows its application in high heat processed products e.g. in baked products (Nofre & Tinti, 2000). It is composed of two amino acids L-aspartic acid and L-phenylalanine and an additional 3, 3-dimethylbutyl group. 3, 3-dimethylbutyl group restrict the peptidase action which would act on dipeptide bond and release phenylalanine, thereby reducing the production of phenylalanine and also the concern of Phenylketonurics. Neotame has been approved by Food and Drug Administration (FDA) since 2002 as a general purpose sweetener for application in foods. However, in India Food Safety and Standards Authority of India (FSSAI) allowed neotame to be used in carbonated water and soft drink concentrate in 2011. Limited information is available regarding the stability of neotame in food products during processing and storage. Therefore, quantitative information on its loss/degradation in food systems is required to be investigated for its safe use.

Due to intense sweetness of neotame, smaller amount is used to sweeten food products. In addition, food complex matrices interfere with the analysis of neotame. Therefore, its determination in foods is an analytical challenge and the development of an analytical method with a high sensitivity is very necessary. HPLC is the most commonly used technique for the determination of neotame in foods (Ji, Sun, Li, Chu, & Chen, 2009; Matsumoto, Hirata, Sakamaki, Hagino, & Ushiyama, 2008). Although HPLC with evaporative light scattering detection (HPLC-ELSD) (Buchgraber & Wasik, 2009; Wasik, Mc Court, & Buchgraber, 2007) and HPLC coupling with electrospray ionization mass spectrometry (HPLC/ESI-MS) (Scheurer, Brauch, & Lange, 2009; Yang & Chen, 2009) have been used, but ELSD detection and mass spectrometry is complex and expensive for routine analysis (Yang & Chen, 2010).

In the present study, a novel analytical protocol based on HPLC with solid phase extraction method was developed for the analysis

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of neotame in cake and ice cream manufactured using this artificial sweetener.

## 2. Materials and methods

Water (PubChemCID:962), Acetonitrile (PubChem CID:6342), Trifluoroacetic acid (TFA) (PubChemCID:6422) and Neotame standard (HPLC grade) was from Sigma–Aldrich (St. Louis, Missouri, USA). Carrez solution no. 1 was prepared by dissolving 3.6 g of potassium ferrocyanide (PubChemCID:71309461) in 100 ml water. Carrez solution no. 2 was prepared by dissolving 7.2 g of zinc sulphate (PubChemCID:24424) in 100 ml water. Neotame (NTM) was procured from Nutra Sweet sweetener Company (Georgia, USA), SPE cartridge Discovery DSC-18LT (6 ml Tube, 1 g) from Supelco (Bellefonte, PA, USA), Maltodextrin from M/S Riddhi Siddhi Gluco-Biols Ltd. (Pantnagar, India), Polydextrose from Danisco India Pvt. Ltd. (Gurgaon, India), Shortenings from Bunge India Pvt. Ltd. (Mumbai, India), Wheat flour from Victoria foods Pvt. Ltd. (New Delhi, India), Corn flour from Weikfield foods Pvt. Ltd. (Himachal Pradesh, India), Whey protein concentrate-70 (WPC-70) from Modern Dairies Ltd. (Haryana, India), Cake gel and baking powder from AB Mauri India Pvt. Ltd. (Maharashtra, India), Vanilla flavour from International flavours and fragrances India Pvt. Ltd. (Chennai, India), Stabilizer and emulsifier mixture Cremodan Sampoorna obtained from Danisco India Pvt. Ltd. (Gurgaon, India).

### 2.1. Equipment

Waters HPLC 515 (Milford, Massachusetts, USA) was used for determination of neotame. It consisted of pump control module II with two Waters 515 HPLC pumps; rheodyne manual injector, temperature controlled column compartment and Waters 2998 photodiode array detector (PDA), Sample loop 20 μl. Chromatograms were analyzed using Empower 2 software.

Solid phase extraction (SPE) vacuum manifold Visiprep™ DL from Supelco (Bellefonte, Pennsylvania, USA), Ultrasonic bath Model-SH-1 from Toshcon Industries Pvt. Ltd. (Haridwar, Uttrakhand, India), pH meter Cyberscan pH Tutor from EUTECH Instruments, Thermo Fisher Scientific (Mumbai, Maharashtra, India), Water activity by Aqual Lab Model Series 3 TE supplied by Decagon Devices (Pullman, Washington, USA), Hobart mixer from Hobart Corporation (Troy, Ohio, USA), Convection oven from HCS Enterprises (Sonepat, Haryana, India).

### 2.2. Cake formulations

#### 2.2.1. Ingredients

The basic formulation is summarized in Table 1. In case of neotame sweetened cake, sugar was completely replaced with most acceptable level of neotame (60 mg/kg cake mix basis). In addition, maltodextrin and polydextrose (50:50) were used as bulking agents.

**Table 1**
Cake formulations.

| Ingredients | Level of ingredients (g/100 g) |
|---|---|
| Shortenings | 20.3 |
| Sugar | 24 |
| Wheat flour | 28 |
| Corn flour | 4 |
| Baking powder | 2.9 |
| WPC-70 | 12 |
| Cake gel | 0.25 |
| Vanilla flavour | 0.8 |
| Water | 8 |

#### 2.2.2. Preparation

All the ingredients required for cake preparation were weighed accurately. Creaming of the shortening was done with a Hobart mixer at medium speed (418 rpm) for 5 min. Sugar was added during creaming, in case of neotame sweetened cake, bulking agents (polydextrose and maltodextrin) were added. The cake gel was added and blended for 2 min to improve the quality of the batter and final product. WPC was added and mixed at low speed (218 rpm) for 3 min. The wheat flour, corn flour and baking powder which were previously sieved together using $12 \times 10^{-3}$ mm sieve size were added and blended continuously at medium speed for 3 min. In case of neotame sweetened cake, as the quantity of neotame was low as compared to other ingredients, hence for proper mixing, it was first mixed properly with water then added to the mix. Finally, water was added and blended at low speed (218 rpm) for 2 min. The batter was filled into pre greased cake molds and was baked at 180 °C for 20 min in a convection oven.

*2.2.2.1. Preparation of ice cream.* Ice cream was manufactured with either sucrose or with neotame by using the method of De (2011) with some modifications i.e. in case of neotame sweetened ice cream, sugar was completely replaced with the most acceptable level of neotame (30 mg/kg ice cream mix basis); 9.9% maltodextrin and 1.5% WPC were used as bulking agent and 0.38% stabilizer and emulsifier were used.

### 2.3. Sample preparation

Twenty gram of cake sample was accurately weighed in a 100 ml beaker. Fifty milliliter of aqueous methanol solution (methanol/$H_2O$, 20:100, ml:ml) was added to it and vortexed for 2 min. It was placed in an ultrasonic bath maintained at 40 °C for 20 min. The solution was then cooled to room temperature (30 °C) and centrifuged at 2000 × g for 10 min. The supernatant was transferred to a 50 ml volumetric flask and added 2 ml of Carrez solution no. 1 followed by 2 ml of Carrez solution no. 2. The solution was shaken vigorously and allowed to stand at room temperature for 10 min. After dilution up to 50 ml with HPLC water, filtration was carried out using Whatman no.1 filter paper. The filtrate was introduced into the SPE cartridge for pre-purification.

In case of ice cream, trapped air bubbles were removed from ice cream by sonication at 30 °C for 20 min. Ten gram of ice cream mix was accurately weighed in a 100 ml beaker and 25 ml of aqueous methanol solution was added. The amount of Carrez solutions no. 1 and 2 were 3 ml each, while the rest of the procedure remained the same as that for cake.

#### 2.3.1. Solid phase extraction

Additional purification was carried out using SPE $C_{18}$ cartridge, previously activated with 3 ml methanol and 20 ml water, as flavourings and fat could not be separated by carrez clarification. 2 ml of the carrez clarified filtrate was added to the activated cartridge. The flow rate through the cartridge was lower than 1 ml/min. The cartridge was then washed with 10 ml HPLC water to remove impurities. Neotame absorbed on the cartridge was eluted with 3 ml of methanol. The eluate was concentrated to dryness by nitrogen drying. The residue was dissolved in 1 ml of methanol and filtered through a 0.22 μm membrane filter. A 20 μl aliquot of the filtrate was analyzed using HPLC.

### 2.4. HPLC conditions

Single column HPLC-UV system: $C_{18}$ (5 μm, 100 Å, 250 × 4.6 mm,