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HPLC METHOD DEVELOPMENT FOR TRACE CONTAMINATION OF PLASTIC POLYETHYLENE TEREPHTHALATE (PET) USED FOR VEGETABLES

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ABSTRACT

A simple rugged and user-friendly and cost effective method to quantify low-molecular-weight formaldehyde and acetaldehyde in the food contact plastic, with 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH) is presented using High Pressure Chromatography (HPLC). Use of 2, 4-DNPH ensure that the probable carbonyl compounds in the food contact plastics, can be monitored using the same method. The separation was achieved with Ascentis Express C18 (100 mm x 4.6 mm x 2.7µ) using Water: Acetonitrile (70: 30 v/v) with a flow rate of 2.0 ml /min. The Method was validated for the analytical parameters viz., Specificity, Limit of Detection (LOD), Limit of Quantitation (LOQ), Linearity and Range, Precision – System, Method and Spiked and Accuracy. For Bottle Gourd, the limit of detection and quantitation was found to be 10 and 30 PPM for formaldehyde with respect to (w.r.t.) sample concentration 10 mg/ ml. The limit of detection and quantitation was found to be 20 PPM and 60 PPM for acetaldehyde w.r.t sample concentration 10 mg/ ml. Primary contact plastic: The limit of detection and quantitation was found to be 250 and 750 PPM for formaldehyde w.r.t sample concentration 0.4 mg/ ml. The limit of detection and quantitation was found to be 500 PPM and 1500 PPM for acetaldehyde w.r.t sample concentration 0.4 mg/ ml.

Keywords – *HPLC, 2, 4-DNPH, Extraction, Plastic PET packaging, Formaldehyde, Acetaldehyde.*

1. INTRODUCTION

Packaging fresh fruits and vegetables is important activity in the long and complicated journey from grower to consumer. There are different packs such as Bags, trays, crates and baskets where recycled PET is extensively used.

The use of recycled plastics for food packaging raises concerns about unregulated substances that may migrate into food and the possible adulteration of the food. In May, 1992, the Centre for Food Safety and Applied Nutrition made available a document entitled "Points to Consider for the Use of Recycled Plastics in Food Packaging: Chemistry Considerations."[1]

Plastic materials are widely used as primary packaging material such as containers, packaging systems, sets, transfer tubing, manufacturing systems and aids and devices[2]. What is migration? Migration is the phenomenon that takes place when chemical substances in the plastic migrate to the surface of the plastic item or to a medium in contact with the item[3].

The migration rate of organic chemical substances depends on their size. Small molecules, typically monomers and residual solvents, will migrate fast as they have a low boiling point. Some monomers such as formaldehyde, vinyl chloride, ethylene and butadiene are all

gases and have a high tendency to migrate quickly even at ambient temperatures and for sure at 100 °C[3]. Larger organic molecules will migrate more slowly, while inorganic metal salts will not migrate. In all cases migration will decrease with time as the concentration of the migrating substances get lower in the plastic. Peroxides and other cross linkers, catalysts and accelerators are used as curing agents in plastics which can lead to migration of formaldehyde[3].

It was observed that the concentration of acetaldehyde in water stored in PET bottles depended mainly on the concentration of acetaldehyde in PET material and could reach more than 200 µg/ml. The temperature, time of storage and concentration of carbon dioxide gas contribute to the migration of aldehydes from bottle walls to mineral water. Higher pressure of the carbonated waters and not CO2 itself or lower pH of waters seems responsible for higher concentration of acetaldehyde[4].

Poly (ethylene terephthalate) (PET) bottles are widely used for beverages. Knowledge about the migration of organic compounds from the PET bottle wall into contact media is of interest especially when post-consumer recyclates are introduced into new PET bottles. An important consequence is that migration levels from PET food-contact materials are largely independent from the nature of the packed food, which on the other hand simplifies exposure estimations from PET [5].

Various substances in plastics used as packaging materials, such as additives and monomers, but also non-intentionally added substances coming from the process or the environment, are of safety concern because they can migrate from the package into food, and also as a consequence of a consumer misuse. Interactions between food and container materials sometimes occur in trace quantities, and such migration needs to be assessed to ensure that it is minimal. Compounds that migrate readily are usually low-molecular-weight and volatile compounds[6].

PET bottles will leach acetaldehyde, formaldehyde, and over eight different PET aromatic fragments when left in the sun for several weeks[7].

Formaldehyde and acetaldehyde are not ICH listed solvents. Its control limit is not available in ICH and USP. Drug regulations have specific and stringent requirements for its registration and usage. There are stringent requirements specifically impurities [8,9]. The daily intake is difficult to evaluate, but a rough estimate from the available data is in the range of 1.5–14 mg/day for an average adult, most of it in a bound and unavailable form [10]. IARC has classified formaldehyde as a known human carcinogen (Group 1) while in United States, it (specifically formaldehyde gas) is classified as reasonably anticipated to be carcinogen by NTP [11]. Absorbed acetaldehyde is distributed in the blood, liver, kidney, spleen, heart and muscle. Regarding repeated dose toxicity of acetaldehyde, oral administration to rats for 4 weeks caused slight hyperkeratosis of the fore stomach at a dose of 675 mg/kg/day. The NOAEL is 125 mg/kg/day[12].

The present work demonstrates a simple extraction method of food and food packaging material followed by simple, sensitive and accurate HPLC method for analysis of volatile trace contaminants, mainly formaldehyde and acetaldehyde in food packaging material by Derivatizing the aldehydes with 2, 4-DNPH. The formation of the derivative was confirmed using HPLC. The chromatographic separation and detection techniques employed could also be applied to the analysis of trace amount of possible leachable carbonyls from pharmaceutical packaging materials.

"As noted in ICH M7 Section 7.5, “Higher acceptable intakes may be justified when human 225 exposure to the impurity will be much greater from other sources e.g., food, or endogenous 226 metabolism (e.g., formaldehyde).” For example, formaldehyde is not a carcinogen orally, so 227 that regulatory limits have been based on non-cancer endpoints. Health Canada, IPCS and US."

EPA (Integrated Risk Information System [IRIS]) 228 recommend an oral limit of 0.2 mg/kg/day, or 10 mg/day for a 50 kg person. 10 mg / day is equivalent to 10000 PPM. LOD of the method is 10 PPM which less than EPA and ICH M7 expectation[13].

Detection limit for acetaldehyde is 20 PPM. Limit of detection for Acetaldehyde by this method is 20 PPM which is far less than current OSHA Permissible exposure level 200 PPM [14].

2.MATERIALS AND METHODS

2.1 Sample and reagents

Bottle Gourd in plastic cover (tray) was bought from market. Sulphuric Acid: AR grade was purchased from Sigma Aldrich 2, 4 Dinitro Phenyl hydrazine: AR grade was purchased from Sigma Aldrich. Formaldehyde: 40% in Water was procured from Merck. Acetaldehyde 100% was procured from Merck. Milli Q HPLC grade water was used in experiment.

2.2 Apparatus and equipment

HPLC analysis was carried out on HPLC Instrument: Agilent 1100 The output signal was processed and monitored using suitable integration software. All studies and separation were achieved on HPLC column, Ascentis Express C18 100 mm x 4.6 mm x 2.7µ

2.3 Preparation of mobile phase

The mobile phase was prepared by mixing HPLC grade Acetonitrile from Merck and Milli Q HPLC grade water. In Mobile phase was sonicated in ultra-sonication bath in (Water: Acetonitrile) (70:30) v/v) and filtered through suitable filter paper.

2.4 Derivatization experiment

Preparation of sample for bottle gourd – 100 gms of recycled Plastic cover (tray)peel was boiled in 100 mL water for 1 hr in round bottom flask attached with condenser. Pipette out 1 mL of this solution in 100 mL of volumetric flask. Add 10 ml of 2% sulphuric acid and 10 mL of 2,4 DNPH and vortex for 1 min. Further dilute up to the mark with diluent.

Preparation of sample for PET bottle Gourd cover (tray) – 4 gms of plastic cover tray cut into pieces was boiled in 100 mL water for 1 hr in round bottom flask attached with condenser. Pipette out 1 mL of this solution in 100 mL of volumetric flask. Add 10 ml of 2% sulphuric acid and 10 mL of 2,4 DNPH and vortex for 1 min. Further dilute up to the mark with diluent.

2.5 Chromatographic condition

Separation was achieved on HPLC column, Ascentis Express C18 100 mm x 4.6 mm x 2.7µ, Mobile phase Water: Acetonitrile (70: 30) v/v with flow rate 2.0ml / min was used. UV detector wavelength was adjusted to 360 nm. Column oven temperature was adjusted to 30°C.

3. RESULTS AND DISCUSSION

3.1 Method development and Column Selection

Different mobile phase and stationary phases were employed to develop a suitable LC method for the quantitative determination of formaldehyde and acetaldehyde. A number of column chemistries supplied by different manufacturers and different composition of mobile phase composition were tried to get good peak shapes and selectivity for the impurities present in recycled Plastic PET tray peel. Peak shape was observed to be poor when Hypersil BDS C18 (250mm x 2.1mm, 5µm) and mobile phase consisting of mixture of 0.1% triethylamine in water: Acetonitrile and Methanol (70:15:15 v/v) formaldehyde and acetaldehyde peak eluted at 12 and 13, however peak shape of formaldehyde and acetaldehyde peak was not good. By using another attempt with mixture of mobile phase 0.1% Triethylamine, Acetonitrile and methanol (60:20:20 v/v) and column Luna C18 (250mm x 2.1mm, 5µm), peak of formaldehyde and acetaldehyde merged and there was no separation. Proper Separation of formaldehyde and acetaldehyde was achieved on HPLC column, Ascentis Express C18 100 mm x 4.6 mm x 2.7µ Mobile Phase Water: Acetonitrile (70: 30) v/v with flow rate 2.0ml / min was used. UV detector wavelength was adjusted to 360 nm. Column oven temperature was adjusted to 30°C.

3.2 Accuracy

Content of Formaldehyde and Acetaldehyde was not detected in Bottle Gourd Sample. Content of Formaldehyde found about (about 309 ppm) and Acetaldehyde below detected limit in plastic cover sample. (refer Table 1)

The accuracy procedure is shown with the recovery studies. The recovery studies were carried out by spiking aliquots of Bottle Gourd sample solution with 100 PPM of formaldehyde and 200 PPM of acetaldehyde. The recovery for formaldehyde was found 94.9 and 102.4% and the recovery for acetaldehyde was found 98.3 and 98.7% (Specimen chromatogram are given in figure 1,2,3,4 & 5).

3.3 Linearity

Linearity standard solutions of formaldehyde derivative (formaldehyde 2, 4 Dinitro Phenylhydrazone) from 30, 50, 100 and 150 ppm. 10 µl solution was injected. The calibration curve derived out of chromatograms for peak area vs concentration of formaldehyde derivative (formaldehyde 2, 4 Dinitro Phenylhydrazone) found to be linear (Refer Graph.1). Observed correlation coefficient was 0.9993.

Linearity standard solution of Acetaldehyde derivative (Acetaldehyde 2, 4 Dinitro Phenylhydrazone) from 60, 100, 200 and 300 ppm 10 µl solution was injected. The calibration curve derived out of chromatograms for peak area vs concentration of formaldehyde derivative (Acetaldehyde 2, 4 Dinitro Phenylhydrazone) found to be linear (Refer Graph.2). Observed correlation coefficient was 0.9999.

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

3.4.1 Bottle Gourd

The limit of detection and quantitation was found to be 10 and 30 PPM for formaldehyde w.r.t sample concentration 10 mg/ ml. The limit of detection and quantitation was found to be 20 PPM and 60 PPM for acetaldehyde w.r.t sample concentration 10 mg/ ml. (Refer Table 2)

3.4.2 Primary contact plastic

The limit of detection and quantitation was found to be 250 and 750 PPM for formaldehyde w.r.t sample concentration 0.4 mg/ ml. The limit of detection and quantitation was found to be 500 PPM and 1500 PPM for acetaldehyde w.r.t sample concentration 0.4 mg/ ml. (Refer Table 3)

Table 1: Content of Formaldehyde and Acetaldehyde in Samples

Content of Formaldehyde and Acetaldehyde in Bottle Gourd & Plastic cover		
	Formaldehyde	**Acetaldehyde**
Bottle Gourd Sample	Not detected	Not detected
Plastic cover Sample	308.55	Below detection

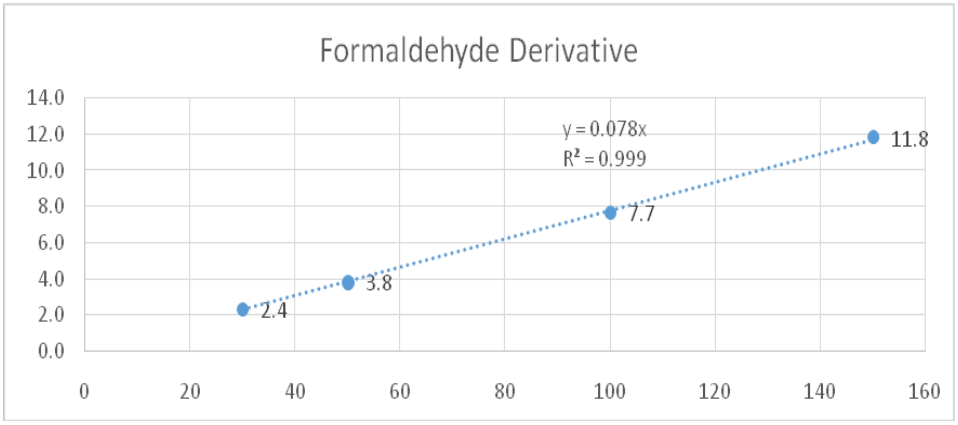
Table 2: LOD & LOQ (Bottle Gourd)

Parameter	**Formaldehyde (in PPM)**	**Acetaldehyde (in PPM)**
LOQ	30	60
LOD	10	20

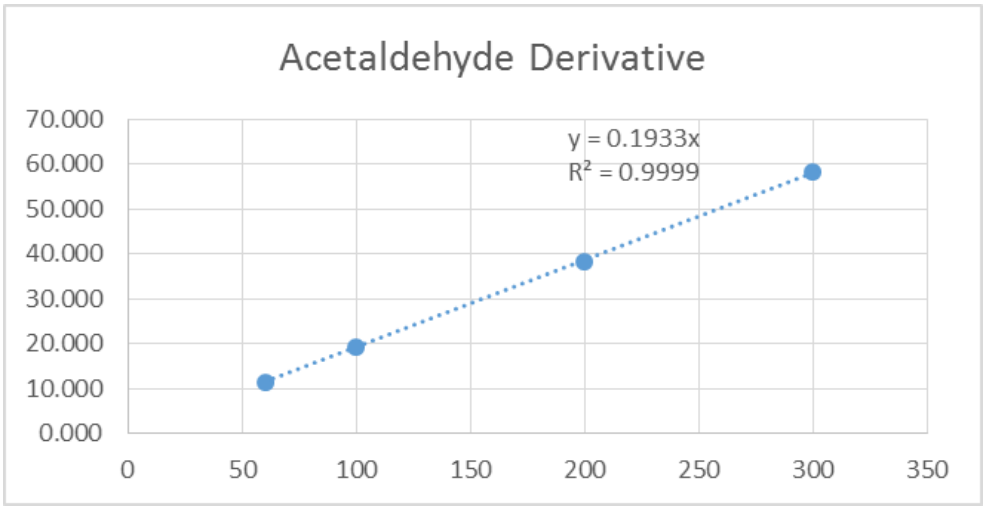
Table 3: LOD & LOQ (Primary contact plastic)

Parameter	**Formaldehyde (in PPM)**	**Acetaldehyde (in PPM)**
LOQ	750	1500
LOD	250	500

Graph 1 : Calibration curve for formaldehyde derivative



Graph 2: Calibration curve for acetaldehyde



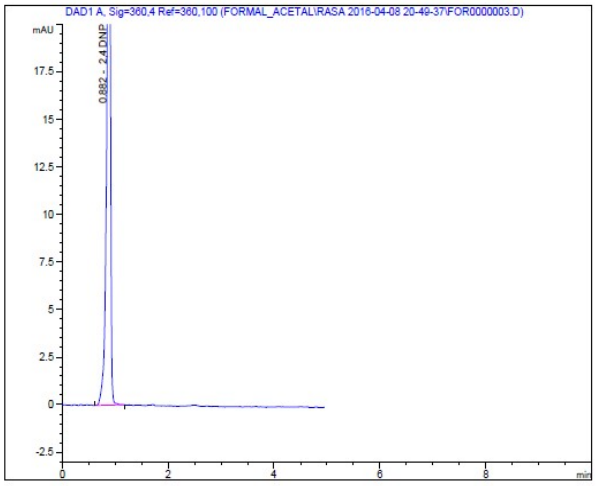


Figure 1: Blank Chromatogram

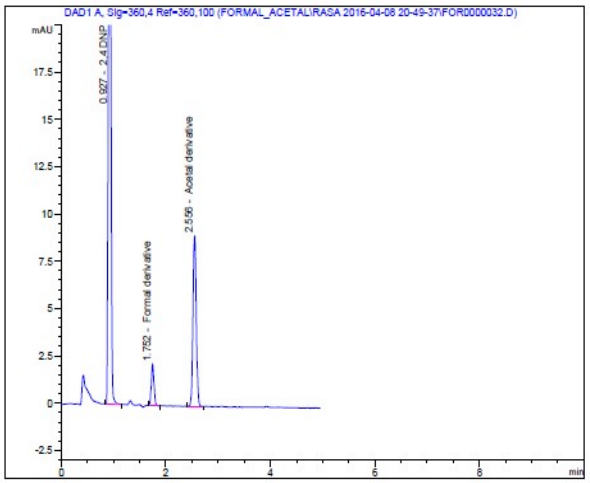


Figure 2: Standard Chromatogram

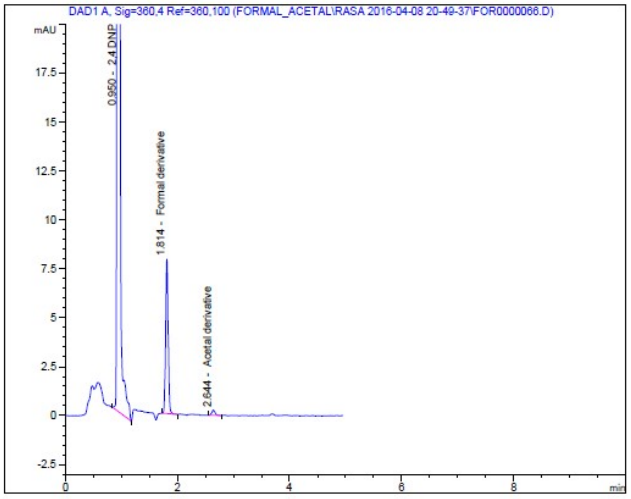


Figure 3: Sample Chromatogram (Plastic cover)

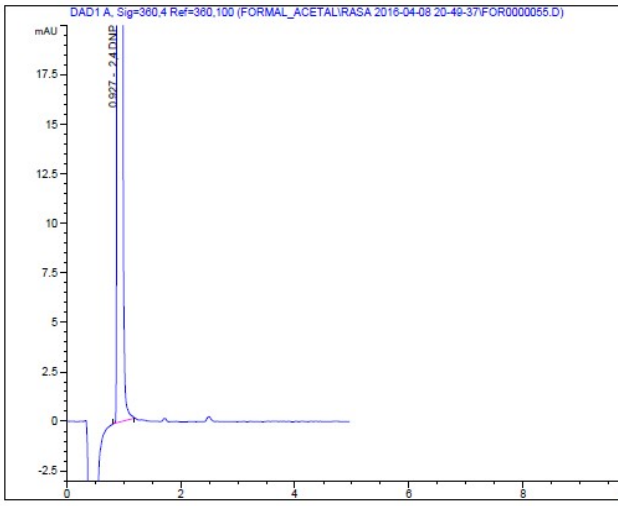


Figure 4: Sample Chromatogram (Bottle Gourd)

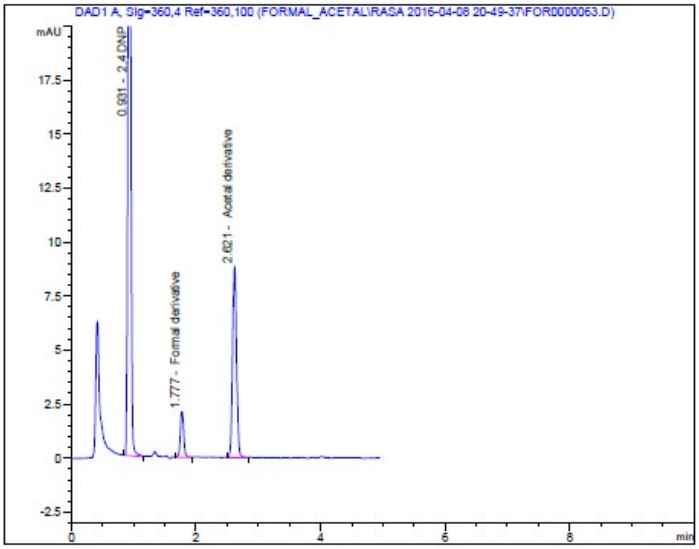


Figure 5: Accuracy (limit 100% level) Chromatogram (Bottle Gourd)

4. CONCLUSION

The proposed LC method is selective for the detection and quantification of Formaldehyde and Acetaldehyde in Bottle Gourd and Primary contact plastic. The method can be used for determination of formaldehyde and acetaldehyde. The method has short run time hence require less consumption of solvent per analysis. Shorter run time would help faster analysis of multiple samples.

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