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Potato Extraction by Using High Performance Liquid Chromatography

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Abstract—*CIPC or chlorpropham (isopropyl 3- chlorophenyl carbamate) is the main sprout inhibitor currently used in potatoes industry. This project involves how the separation is done using HPLC, it is referred as High performance liquid chromatography. An HPLC- UV method was developed and validated for the separation and quantification of this compound using propham as an internal standard (IPS). The proposed HPLC method was composed with the standard gas chromatography of the CIPC residue extracted showing good agreement (R_2 =0.99). Despite using the same extract the recovery results for the proposed HPLC method were 13% higher than GC analysis.*

Keywords:- *Gas chromatography, CIPC, Soxhlet Extraction.*

1. INTRODUCTION

Chromatography is a technique for the separation of a mixture by passing it in solution or suspension through a medium in which the components move at different rates. The modern form of column chromatography is called as high performance liquid chromatography, high resolution, high speed liquid chromatography. High performance liquid chromatography is a branch of liquid chromatography, primary HPLC is method use for separation, categorize and quantify each component in a mixture liquid chromatography where ineffective because of flow rate of solvent being depend of gravity. We know that separation is very slow process and that depend upon method which was used for separation. Gas chromatography is more effective method than liquid chromatography but gas chromatography was not useful for separation of biochemists because instability of solutes. So finally, scientist were design HPLC. The partially HPLC process is similar to liquid-liquid extraction but it is continuous not batch wise. The composition of mobile phase which is also called as eluent that depends on intensity of interaction between the components. The development of HPLC is primarily about evaluation of particle technology. In column chromatography, mobile phase forced through column that result into decrease the analysis time by 1-2 order of magnitude that relative to classical liquid chromatography, because of reduction in analysis time, be the use of much small particle of the adsorbent becomes possible and that results into increasing the column efficiency. There is very difference in method of HPLC and LC. The operating pressure of HPLC is about 50-350 bar which is higher than liquid chromatography whereas liquid chromatography build on force of gravity. HPLC method build on pumping system that pass pressurized liquid and mixture of sample through the column, the column is filled with various adsorbent that may result into separation of sample .The separation of component is done only because of different degrees of interaction with the adsorbent particle. In HPLC use pressurized liquid that is a mixture of many solvent (like water, acetonitrile and methanol). HPLC dealing between two phase in which first phase is mobile phase and second phase is stationery phase. The composition and temperature of mobile phase plays vital role in separation during interaction takes place between sample and adsorbent. These interaction are hydrophobic, dipole-dipole, ionic. HPLC has been used for manufacturing (e.g. during the production process of pharmaceutical and biological products), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other),and medical (e.g. detecting vitamin D levels in blood serum) purposes. The chromatography is performed by exact differentiation, selective identification and quantitative of structurally closely related compounds.

2. METHODS USED

Reagent like Chlorpropham (95%), 3-chloroaniline (99%), propham (IPC) (99%), Methanol (HPLC grade) ,Stock solutions of 10000μgmL−1 of each compound (CIPC, IPC, and 3-CA) were prepared in methanol. These individual stock standard solutions were stored in a refrigerator at 4∘C and were used to prepare the working solutions at different concentrations. The standard solutions were warmed to room temperature of 20°C before injecting. The HPLC system consist of autosampler, pump flow rate of 1.5mL/min and a injector with volume of 20µL along with a column at ambient

temperature & UV 100 detector coupled with Dionex Peaknet software.

The mobile phase in this was prepared by analysis the methanol & water helium gas was used in HPLC for degassing the mobile phase. Here the mobile phase was prepared one day before analyzing ,kept for 20ºC at fixed room temperature.

When the mobile phase is passed through the HPLC column chloropropham (CIPC) ,propham (IPC) along with 3-chloroaniine(3-CA) is been separated inside it .This separation in the column take place due to difference in there concentration ,polarity or solubility. Hence we can say that In an HPLC System separation take place on the base of the concentration, different strength of the Mobile phase for achieving the better chromatogram various concentration of Methanol has been taken. Such as 70%, 60%, 55%, 50% concentration of methanol Mobile phase have been tested in HPLC. The injector 5 replicate injections of 20µL containing mixture of CIPC, IPC & 3-CA were injected in the HPLC column, simultaneously there is flow of the Mobile phase in the Column. Hence there is the main part were separation of this there mixture take place due to difference in concentration. Later we get the chromatogram with help of UV100 Detector used at detection wavelength of the 210nm. The calibration curve of different compound in mixture as detected and shown on the graph in the software.

3. EXTRACTION OF CIPC

For prove various parameters of HPLC System analysis of CIPC is done by treating the peels of potato that consist of the CIPC .CIPC is the main sprout inhibitor used by potato industry. Along with IPC & 3-CA. The Soxhlet extraction procedure was applied to extract CIPC from treated potatoes. Particularly 30 potatoes tubers consist of CIPC were chosen from large commercial stores. After washing and drying procedures were performed, the peel from each tuber was placed into a cellulose thimble, which contained 10g of the drying agent sodium sulphate to remove the water from the potato peel. The thimble was plugged with cotton wool and placed into a Soxhlet extraction unit prior to extraction with 150mL of hexane solvent. The peel was extracted for approximately two hours after the first reflux. The extract in the round bottom flask was quantitatively transferred to a 100mL volumetric flask and made up to volume. Then Analyzing by HPLC was done. Before analysis extract was filtered through 0.2µm Teflon membrane syringe filter in HPLC.

4. CHROMATOGRAM

The peaks of eluted compounds were identified in the chromatogram through a comparison of the retention times based on an analysis of a standard mixture and individual reference standards. The peaks of 3CA and IPC appeared first and second, respectively, before the final peak of CIPC. However, the impurity peak has little effect on the background of the baseline of the 3-CA peak. The source of the impurity peak might be caused by the methanol solvent; it was present in small amounts. The peak height of propham was quite small due to its absorbance being very low at a wavelength of210nm.

5. RESULT

The chromatographic an conditions were set based on isocratic method using methanol/water as the mobile phase. Propham (IPC) was chosen as the internal standard due to its similarity in structure to chlorpropham with the only difference being the absence of one chlorine atom in the phenyl ring (Figure1).

This test exhibited good UV absorbance for all compoundsatawavelengthof210nm, although the peak height of propham was quite small due to its absorbance being very lowatawavelengthof210nmcomparedwithitsλmax200nm. The separation between the compounds was dependent on their polarity. Because of the wide range of polarities between these compounds ,the higher polarity compound was eluted first from the HPLC column For that reason ,the peaks of 3CA and IPC appeared first and second, respectively, before the final peak of CIPC.'

6. CONCLUSION

CIPC is a compound of the well-known group of N phenyl carbamates which may undergo rapid degradation under unsuitable solvent and excessive heating conditions releasing 3-CA [1–5]. For public health and environmental consideration, there is concern about their residues [6, 7]; hence, analytical methods are required to analyze the residues of these phenyl carbamates in potato and environmental samples particularly CIPC and its degradation product 3chloroaniline3-CA.This project was undertaken during a global shortage of acetonitrile in 2008. The proposed method showed results for CIPC residues that were approximately13%higher than GC method.

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