HPTLC ANALYSIS OF FOODS IN INDIA

By
Dr.P.K.Jaiswal, M.Sc.,D.Phil.,D.Sc.,F.I.C.
Director of Laboratories
Central Agmark Laboratory
Govt.of India
North Ambazari Road
NAGPUR-440 010
INDIA
e.mail: jaiswalp_ngp@sancharnet.in

Introduction

- High Performance Thin Layer Chromatography became one of the important tools capable of giving fast results, high resolution, and separation of more accurate and precise quantitative results with many advantages over other techniques. Thin layer chromatography is widely used for food analysis and quality assessment throughout the developing world.

Applications

- In the domain of food composition, for verification of food labeling, additives, adulterants, contaminants, decomposition related to determinations of compound classes such as amino acids (protein for determination of quality), lipid and fatty acids (for evaluating quality and adulteration in oils/fats), sugar (beverages), biogenic amino acids (for assessing storage stability), vitamins, nutrients, colorants, antioxidants, preservatives, special quality factors and carbohydrates.

ABOUT AGMARK LABORATORY, INDIA

- Engaged in formulation and review of quality standards of agricultural and food commodities, standardization and development of methods etc.

- Studies have been carried out in method validation/ determination of aflatoxin in different commodities, determination of Argemone oil and Mineral oils in other oils etc. by HPTLC.

- Advantages of HPTLC in food analysis and contaminants
- Unified food law in India
- Deliberate adulteration in food for economic benefit
- Contamination in Food

- TLC in the past for detection of adulteration
- Standardization and Validation of method available with TLC and replacing with HPTLC for detection/quantification at a much lower level

- Detection of rice bran oil in other edible oils, detection of mineral oils in other vegetable oils, food commodities etc.

- Detection of animal fats in vegetable fats, Detection of Castor oil in edible oils, and its differentiation from rancid oils

- Detection of Karanja oil in other edible oils

- Detection of toxic tricresyl phosphate and determination of tri-o-cresyl phosphate in edible oils
- Identification and quantification of oil soluble colours

- Detection of Kesari dal/flour in edible dals/besan
- Detection of true cinnamon
- Detection of colophony resins in asafoetida, Diazepem in toddy, chloral hydrate in toddy.

- Food preservatives - Class I and Class II
- Maximum permissible limits for Class II
- TLC most simple and convenient techniques
- Detection of benzoic acid and sorbic acid, p-hydroxy benzoate and its esters, volatile acids such as propionic acid and acetic acids etc. in food

- Detection and determination of aspartame, acesulfame, saccharin, cyclamate, natural food colours, permitted coaltar food colours.

- Detection of antioxidants like BHT, BHA, TBHQ in fats etc., detection of flavouring agents like vanillin, ethyl vanillin and coumarin.

Food Contaminants

- Contaminants in food due to negligent handling, processing, storage, transportation etc.
- These toxicants may be injurious to health at a low level and thus constitute a part of food safety quality assurance system.

- Due to liberalization of world market, maximum permissible limits of these contaminants play an important role in marketing of food from one country to another country or even within the country.

- Analysis of many pesticides residues using TLC has been a good official method in the past and lost its significance in view of its reliability and detection limits.

- Considerable work has been carried out for screening of many pesticides at a time using HPTLC.

- Screening of more than 250 pesticides in water sample is a landmark work and road map for exploiting such techniques in screening of pesticides in different food samples at a better accurate and acceptable level of detection and quantification.

- Such methods would, no doubt, be not only cheaper but convenient also and interpretation would also be easy as compared to other hyphenated techniques.

- Analyst would have to validate the method for different food and different pesticide keeping in view sampling, extraction and recovery.

- Application of HPTLC for detection/determination of Aflatoxin, mycotoxins is an inspiration for use of this instrument, as an official/validated method for determining and screening of pesticides residue in food to re-establish the importance of planer chromatography in the domain of pesticide residue in food.

- Presence of heavy metals contaminants which enters the food through water, air, industrial pollution, agricultural technology, use of utensils during processing of foods etc., make food toxic and unfit for use.

- Maximum different permissible limits have been prescribed in various foods under several national and international food laws.

- These metals constitute lead, copper, arsenic, zinc, tin, cadmium, mercury, methyl mercury, nickel and chromium.

Important Applications of HPTLC in India in Food Analysis

Quantitative Determination of Sudan – I,II,III,IV etc. In Chilli Powder / Chilli Whole.

- Measurement mode : UV absorbance/ reflectance
- Quantification scanning – 254 nm and 500 nm
- Detection level :

I – 11.0 ng/spot

II – 9.9 ng/spot

III – 9.0 ng/spot

IV – 8.6 ng/spot

- LOD for other coaltar dye

Methyl yellow	- 10.0ng/spot
Para red	- 9.5ng/spot
Rhodamine B	- 8.0ng/spot
Sudan Orange G	- 8.6ng/spot

Quantitative Determination of Food Colours permitted in food

- Max. limit of permitted food colour in India
- Quantitative determination of Ponceau 4R, Carmoisine, Erythrosine, Tartrazine, Sunset yellow FCF, Indigo carmine, Brilliant blue FCF and Fast green FCF in food was done at ppb level.
- Measurement mode UV absorbance

- Visualization – 254 nm and 366 nm for quantification
- UV absorbance/reflectance and Spectra recorded for identification at 190-800 nm
- Measurement in UV absorbance mode
- Scanning : 254 nm and 366 nm for quantification

Quantitative determination of Amaranth and Allura Red(Banned)

- Measurement mode : UV absorbance/ reflectance
- Visualization under UV cabinet 254 nm and 366 nm
- For identification record spectra between 200 – 400 nm and match with standard
- For quantification scan at 520 nm

Quantitative Determination of Cholesterol in Edible Oil

- Cholesterol in edible oil – UV absorbance/ reflectance mode
- Quantification : 200 nm and 600 nm
- Identification : Record spectra between 190 to 400 nm
- Confirm match with standard at Rf 0.14

Quantitative Determination of Nicotine in Tobacco

- Quantitative determination of Nicotine : 0.4-0.5 µg
- Quantification: Scan at 262 nm
- Record Spectra between 190-400 nm
- Confirm match with standard at Rf 0.58

Quantitative Determination of Saffron in Food

- Scanning for quantification : 254 nm and 430 nm before derivatization and at 580 nm after derivatization
- Measurement mode : UV absorbance/ reflectance
- Post chromatographic derivatization is done by dipping the plate in anisaldehyde sulphuric acid and then heated to 110 ^{0}C for 10 minutes

Quantitative Determination of Caffeine in Coffee/Tea

- Measurement mode: UV absorbance/ reflectance
- Quantification scanning : 275 nm
- Record spectra : 190nm – 400 nm
- Confirm match with spectra at Rf 0.18

Quantitative Determination of Castor oil in Edible Oil

- Measurement mode: UV absorbance/ reflectance
- Post Chromatographic derivatization by dipping the plate in 10% methanolic sulphuric acid solution. Heat to 110 ^{0}C for 10 minutes.
- Visualization in UV cabinet at 366 nm
- Quantification: Scan at 366 nm

Quantitative determination of Eugenol in Clove Oil/Clove

- Post chromatographic derivatisation is done by spraying the plate with 1% ethanolic vanillin solution and then the plate is dipped in a 10% ethanolic sulphuric acid, there after plate is heated to 110 ^{0}C for 10 minutes.

- Measurement mode UV absorbance/reflectance

- Quantification : Scanning at 285 and 580 nm
- Identification : Record spectra between 190 to 400 nm
- Match with standard

Quantitative Determination of Preservatives (Methyl paraben, Propyl paraben, Sodium benzoate) in Tomato Sauce

- Measurement mode UV absorbance/ reflectance
- Quantification : Scanning at 227 nm and 254 nm
- Identification: Record spectra 190 nm to 400 nm
- Confirm match with standard

Quantitative Determination of Chicory in Coffee

- Measurement mode UV absorbance/ reflectance
- Quantification : Scanning at 275 nm
- Identification: Record spectra of marker Rf 0.30
- Confirm match with standard between 190 to 400 nm

Quantitative Determination of Formaldehyde in Milk

- Samples requires pre-chromatographic derivatisation with dimedone in methanol as formaldehyde is volatile
- Measurement mode UV absorbance/ reflectance

- Quantification : Scanning at 254 nm
- Identification: Record spectra between 190 nm to 400 nm
- Confirm match with standard

Quantitative Determination of Piperine in Piper Nigrum

- Measurement mode UV absorbance/ reflectance
- Quantification: Scanning at 334 nm
- Identification: Record spectra 190 nm to 400 nm
- Confirm match with standard Rf 0.38

Quantitative Determination of Antioxidants (Butylated Hydroxy Anisole (BHA) & Butylated Hydroxy Toluene (BHT), Tertiary Butyl Hydroxy Quinone (TBHQ) in edible oil

- Post chromatographic derivatisation has been done by dipping the plate in phosphomolybdic acid and then heated 110 ^{0}C for 10 minutes.

- Measurement mode UV absorbance
- Quantification: Scanning at 200 nm and 285 nm
- Identification: Record spectra 190 nm to 400 nm
- Confirm match with standard

Quantitative determination Amino Acids in Potato

- Scanning is done by fluorescence and limit of determination are in nanogram range.
- Different amino acids like arginine, threonine, glycin, alanine, phenylalanine, tryptophan, valine, leucine were determined.
- Scanning by fluorescence at 313/460 nm.

Identification and quantification of sugar in beer and wine

- Post chromatographic derivatisation was done by drying the plate and heating it at 150 ^{0}C for 4 minutes.
- Thermal reaction is usually monitored under UV 366 nm.
- Possible to determine low nanogram range with scanning by fluorescence with mercury lamp with at 366/> than 400 nm.

Determination of Aflatoxin

- Aflatoxin in food as contaminant is very toxic and low level of 30 ppb has been prescribed under mandatory food law .

- Assessment of Aflatoxin in samples collected from market for groundnut, mustard oil, ghee, anardana, ajwain, clove, coconut, amla whole, amla powder, castor seed, shikakai, mahua and tamarind were carried out.

S. No	Name of Commodity	No.of samples	No.of samples reported positive	Range of aflatoxin in ppb found
1	Amla whole (dried)	38	12	0.2-4.0
2	Amla powder	15	11	0.2-6.2
3	Castor seed	49	21	0.2-5.7
4	Shikakai	34	24	0.5-9.0
5	Tamarind	59	3	0.1-0.7
6	Anardana	60	26	0.5-14.1

S. No	Name of Commodity	No.of samples	No.of samples reported positive	Range of aflatoxin found
7	Ajwain	53	5	0.8-1.9
8	Clove	38	25	0.4-9.6
9	Coconut	55	4	0.3-2.2
10	Mustard oil	97	7	0.5-5.8
11	Ghee	87	1	1.1
12	Groundnut	51	10	1.16-108.0
13	Mahua	42	3	0.3-0.9

Quantitative Determination of Argemone Oil in Mustard Oil

- The hydrochloric acid extract of the oil samples containing Argemone oil when subjected to chromatography for separation of alkaloid gives fluorescent spot under UV at 254 nm.

- Similar work for quantitative evaluation of Sanguinarine as an index of Argemone adulteration in edible mustard oil have also carried out to assess the contamination of edible oil by Argemone oil especially in poisonous cases due to Argemone toxicity.

Quantitative Determination of Lactose, Saccharose and fructose/glucose, Mono-Di, Tri- and Polysaccharides in various foods

- Post chromatographic derivatization of the sample was done by dipping the plate for 3 seconds in diphenyl amine reagent.
- Plate is heated under 120 ^{0}C for 10 minutes.

- Using NH_2 plates,the sugars fluoresce simply by heating the plate at 130 ^{0}C for 5 minutes.

- Scanning is done by absorbance at 620 nm or fluorescence at 366/>400 nm with mercury lamp.

Determination of Phospholipids

- The lowest detection level is nanograms.

- After post chromatographic derivatization, quantification is done by absorbance at 550 nm or by fluorescence.

- Post chromatographic derivatization was done by immersing the plate for 5 seconds in a solution (0.4 gm manganese chloride in 60 ml of water, add 60 ml of methanol then 4 ml conc.sulfuric acid. Cool the solution).

- Phospholipid fractions of about 100 ng appear as brown zones.
- Under 366 nm UV fluoresce, enabling determination limits of 10 ng.
- Densitometric evaluation was done by scanning by absorbance at 550 nm tungsten or mercury lamp and scanning by fluorescence with mercury lamp at 366/>400 nm.

Quantitative determination of Vitamin C in fruit juice

- Mild oxidation converts ascorbic acid to dehydroascorbic acid.
- Its 2,4 dinitrophenylhydrazone derivative is then separated from matrix substances and quantified densitometrically by absorbance at 510 nm.

Conclusion

- This laboratory has been engaged in validation of methods
- Not much work has been carried out, especially with regard to method validation, measurement of uncertainties. Dynamic range-range of quantification, accuracy, applicability, practicability, repeatability standard deviation, reproducibility standard deviation, ruggedness, sensitivity, specificity, trueness, LOD and LOQ etc.

- Extensive collaborative studies are undertaken in the application laboratories so that single instrument will make possible to have a multifarious and varied application in foods.

- HPTLC can, thus, be established as a unique tool for screening, detection and determination of different food contaminants, preservatives, antioxidant, adulterants, establishing the food composition and food labeling facts finding tool.

- Such techniques would no doubt be cheaper, convenient, easy to handle and affordable in an analytical laboratory in our country where presence of food contaminants in agricultural and food commodities are threat in assessing the quality and safety of foods.

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