DETERMINATION OF CHLORAMPHENICOL IN DIFFERENT COMMERCIAL SAMPLES OF HONEY

Raja Shekhar **Nunavath***, Ajaykumar **Chittipolu,** Prithviraja **Gajjela**

Department of Pharmaceutical Analysis, University College of Pharmaceutical Sciences, Warangal-506009, Telangana, India
E-mail: sheshiraj7@gmail.com

Abstract

Honey is a natural product produced by honey bees. Due to its health benefits and huge demand in market production of commercial honey has been expanded. It is necessary to look at the quality and contamination of such products whether they are in limits or not according to the Bureau of Indian Standards. Contamination may cause by antibiotics used to treat the honey bees in apiculture, Chloramphenicol is one of the major contaminants in apiculture. Monitoring the chloramphenicol in honey helps to access the risk of their products to consumer health and gives information on the chloramphenicol levels in honey determined by HPLC method of analysis. Total four branded products from the Indian market and one natural honey sample from a rural area of Telangana state, India were selected for the analysis in which two compounds shows chloramphenicol residues. As per the European Union, the minimum residue levels (MRL) of Chloramphenicol in honey is 3ppm/kg. However, the found concentrations are under MRL levels, can be concluded that the honey products from different brands having low levels of chloramphenicol levels.

Keywords: Honey, honey bee, apiculture, antibiotics, Chloramphenicol, HPLC, MRL levels.

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1. INTRODUCTION

According to The Codex1 Alimentarius Commission defines honey as "'the herbal candy substance produced by way of honeybees from the nectar of flora or secretions coming from residing organisms feeding on plants, that bees gather, seriously change and mix with precise ingredients, store and depart to ripen in the hive". According to the Bureau of Indian Standards (BIS) specs IS 4941:1994, honey is described as "natural candy substance produced by using honey bees from the nectar of blossoms or from secretions of flora which honey bees collect, transform and store in honeycombs for honey bees".

In spite of being a natural and safer food, honey has long been held in high regard. Today, though, honey is manufactured in an atmosphere contaminated by numerous forms of pollution. The causes of pollution can be biological, microbial, apicultural, pesticidal (chloramphenicol, organochlorine). In honey, antibiotics are primarily found when they are used in apiculture to cure bacterial diseases.[1-2]

Honey is currently regulated under three legislations:

Food Adulteration Prevention (PFA) Regulations, 1955, a mandatory norm enforced by the Authority for Food Safety and Standards 3. Under IS4941:1994, the voluntary *Bureau of Indian Standards (BIS)* norm for extracted honey. It would have to be pursued by brands wishing to receive the ISI mark.

Enacted by the Union Ministry of Agriculture, Honey Grading and Marking Regulation, 2008, under *the Agricultural Produce (Grading and Marking) Act, 1937 (AGMARK).*

Honey is classified as a "natural good" by all the above three laws and sets standards for its structure and consistency (such as sucrose content, total sugar reduction and moisture content), but there are no standards for honey antibiotics.

Indian authorities feel they can't regulate as there are no requirements. But when it comes to honey for export, this perception is subject to adjustment. For this reason, an extensive surveillance mechanism (called the Residue Monitoring Plan or RMP) has been placed in

place and the role of regulating exports has been assigned to the Exports Inspection Council (EIC) under the Ministry of Trade and Industries of the Union.

EIC standards:

There are numerous kinds of tainted honey products that have been recorded several times worldwide. Chloramphenico[4-5],organochlorine6[-9], carbamate, pyrrolizidine[10], polycyclic aromatic hydrocarbons[11] and other pesticides[12, 13,] heavy metals[14], such as Ag, Pb, Cu, Cd and microorganisms, are the main pollutants.

For honey, which is shipped, the EIC has set antibiotic requirements. This is referred to as the 'Standard of Action (LOA)' cap above which a sample is assumed to be non-compliant for exports and withdrawn. These LOAs have been set for antibiotics; oxytetracycline (10 ppb) and chloramphenicol (300 ppb) are set for LOAs, for instance.

For honey sold domestically or as regulations for imported honey, these laws do not apply (Codex Alimentarius Standard for Honey, 1993). Over the last few years, the EU Member States' food and feed management authorities have found Indian honey tainted with antibiotics such as chloramphenicol. Indian honey is tainted with tetracycline and streptomycin in US exports too though.

The Ministry of Commerce and Industries of India has developed a Residue Monitoring Plan (RMP) to monitor the extent of contamination of antibiotics, heavy metals and pesticides in honey intended for export, in order to facilitate the export of honey. It is very evident that India has developed an extensive framework for controlling the output of honey exported to the EU and the US. For the domestic market, though, there is no standard for antibiotics in honey.

On the antibiotic contamination of honey consumed within the region, there is hardly any report. Similarly, honey9 is also imported by India, but there is no standard to verify the consistency of imported honey. To fill this gap, this study was undertaken. Therefore, the purpose of this analysis is to figure out the amount of antibiotics present on the domestic market in honey samples.[2]

Benefits of honey: Honey bee and honey products are good biological indicators because they indicate environmental pollution through two signals one by showing mortality rate due to pesticides and other by showing metal residues in their bodies Honey is containing a wide range of vitamins (pantothenic acid, riboflavin, niacin), minerals (calcium, copper, manganese, magnesium, phosphorous, potassium),antioxidants, amino acids, scavenging or eliminating free radicals, wound healing property.[3]

2. MATERIAL AND METHODS

2.1. Materials:

SAMPLE NAME	BRAND NAME
Sample A	Dabur honey
Sample B	Patanjali honey
Sample C	Reliance honey
Sample D	Lion honey
Sample E	(Natural Honey)

2.1.1. Equipment

Table-1 Equipment's used in present work

Equipment	Manufacturer
Refractometer	Sisco Research Laboratories Pvt Ltd
HPLC	Shimadzu Corp. Manufacturing company
UV	ShimadzuCorp.Manufacturing company
pH Meter	ElicoLtd
Centrifuge	Remi
Cyclo Mixer	Remi

2.1.2. Chemicals

Chemicals used in present work Methanol, Ethyl acetate, Carbon tetrachloride (CCl4), Glacialacetic Acid, Hexane, Orthophosphoric acid, Acetonitrile were purchased fromMerck& Co. Pharmaceutical Company.

2.2. Methodology

2.2.1. Refractometer method of analysis

Measuring the changes in refractive index or moisture content[24-25]of column effluent passing

through the flow cell, greater the difference in the refractive index between sample and mobile phase greater will be the imbalance and the sensitivity is also higher. In case of complex mixtureswhich cover the wide range of refractive index values becomes difficult to detect in the detector. This factor is severely limiting RI detector application in the analyses requiring the gradient elution, where mobile phase composition is changed during the analysis to affect the separation.[16-17]

2.2.2.Sample extraction[18]

Accurately weighed 20gms of each study sample (A, B, C, D and E) taken into centrifuge tubes separately. To the above samples, 10 ml of Ethyl acetate was added to each tube and stirred for 10min on a Vertex shaker. After stirring on Vertex shaker the upper Ethyl acetate layer was transferred in a clean Turbo van tube. Repeated the extraction process with another 5ml of Ethyl acetate and collected the Ethyl acetate and dried under Nitrogen. Dissolved the dried residue in 1ml of Hexane: carbon tetrachloride (1:1v/v) and 1ml of water added, mixed properly by usingvertexing. To separation of layers, the above mixture Centrifuged for 15minets (Food and agricultural immunology, 2016**).** [20-21]

2.2.3.HPLC method of analysis

Chromatographic conditions: [20]

Flow rate: 1ml/min
Column:Kromasil 250 x 4.6 mm, 5µ(C_{18})
Wavelength: 278nm
Column temperature: 25°C
Injection volume: 10µL
Run time: 8min
Diluent:Water and Acetonitrile (70:30).
Type of elution:Gradient elution
Detector: PDA

MOBILE PHASE OPTIMIZATION

Buffer (0.1% OPA)[19]:1ml of orthophosphoric acid solution in a 1000ml of volumetric flask added about 100ml of milli-Q water and final volume was made up to 1000 ml with milli-Q water.

Mobile phase: Buffer and Acetonitrile taken in the ratio 50:50 (OPA: Acetonitrile) [19.]

3. RESULTS AND DISCUSSION

3.1. Results

The detection of Chloramphenicol residues in varies brands of honey samples (Sample A, B, C, D&E) carried out by using the HPLC method with a PDA detector. The detection wavelength was chosen at 278 nm because the taken drug CAP is absorbed highly at this wavelength. CAP was used as an internal standard to examine and compare each sample taken for the presence of CAP in the honey brands in the Indian market.

The taken all brands of samples R.I values was carried out by using Refract meter analysis because the value of the refractive index can be influence the column and also the detector sensitivity. The refractive index values of the all samples were within the standard values then the sample preparation carried out for HPLC analysis and the taken samples were dissolved in ethyl acetate because honey can soluble easily in ethyl acetate. The mixture transferred to vertex shaker for separation.

Table-2: Refractive index values of samples

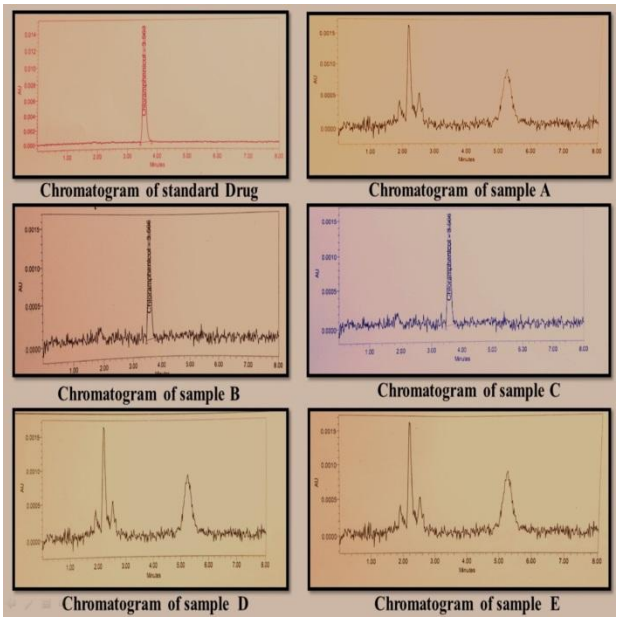
Sample	R.I (Temp. 20°C)	R.I (Temp. 25°C)	Percentage of moisture
A	1.483	1.484	21.4%
B	1.481	1.482	22.0%
C	1.483	1.484	21.4%
D	1.484	1.485	21.0%
E	1.480	1.468	22.0%

Temperature correction for refractive index =0.00023°C.

(Indian standard extracted Honey-specification (2nd revision) IS 4941:1994-1974)[26]

Table-3: Results of honey samples by the HPLC method for evaluation of CAP

Peak Name	RT	Area	USP Plate Count	USP Tailing
CAP STANDARD	3.566	2457	6323	1.31
Sample-A	3.565	0	0	0
Sample-B	3.566	980	5947	1.24
Sample-C	3.564	978	8721	1.34
Sample-D	3.565	0	0	0
Sample-E	3.566	0	0	0



Chromatograms of standard drug CAP and other honey samples

The above table shows the quantity of the Chloramphenicol present in the taken samples in parts per million levels. The MRL (Maximum Residue Level) as per European Union is 3ppm/Kg. We took European Union standards because in India there are no specific regulations for honey adulteration, especially for chloramphenicol adulteration. As per the table3, the two samples were detected the presence of chloramphenicol.

3.2. Discussion:

The main aim of this study is the determination of Chloramphenicol residues in selected marketed commercial Honey products The above results elucidate that the samples B and C contains Chloramphenicol residues. Sample B found 0.0114 ppm/g and 0.57 ppm/kg and sample C found 0.0088 ppm/g, 0.43 ppm/kg. The remaining samples A, D, and E were not traced for any Chloramphenicol residues.[22]a severe health concern can arise from the long-term ingestion of some sort of unnecessary adulterants. In this inquiry, the traced adulterants were overlooked by the Indian regulatory authority because there were no clear rules for the processing of honey due to this Indian honey was barred throughout Europe by the European Union[23, 24] . The above analytical method, however, was not validated for further analysis, but the presence of chloramphenicol was confirmed by the primary analysis of two of the five marked honey samples taken. It would be very beneficial for the regulatory body and quality management initiatives for commercial honey if a quick, rapid and cost-effective method was prepared and tested to detect the CAP in honey samples.

4. CONCLUSION

Currently the way of approaching for a healthy and happy life has changed; people prefer natural methods to improve their immunity. Honey acts as a natural antibiotic due to its astounding health benefits urge for honey consumption has risen across the globe, which creates high demand subsequently increase malpractice in production and marketing honey products. By Performing HPLC method analysis for samples A, B, C, D and E found that the residues of Chloramphenicol were detected in B and C honey samples. However, the level of residues CAP is within the limits (0.5µg/Kg, 0.43µg/Kg) but daily consumption of contaminated honey will cause health disorders (Examples: Antibiotic resistance, multi-organ failure etc.)

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Conflict of interest:
All the authors declare that they have no conflict of interest.

Abbreviation used:
All the abbreviations used have been described in the text.

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