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Research Article

COMPARATIVE EVALUATION OF ENDOSULFAN CONTENT IN TRIPHALA CHURNA MARKETED IN YAVATMAL DISTRICT OF INDIA BY HPLC METHOD

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ABSTRACT

In the present study herbal products marketed in Yavatmal District of India were determined for the presences of Endosulfan contents in herbal products were examined. The total of ten herbal products of various brands were selected randomly and tested for pesticide content. Of which 2 samples (H3 & H4) showing the presence of Endosulfan but within the limit given by W.H.O. The formulations are used daily by the patients suffering from constipation. The method reported was used for analysis of pesticide content in the present work for determination of Endosulfan content in Triphala Churna formulations in which chromatographic conditions were mobile Phase 0.1% acetic acid in Acetonitrile Flow rate 1 ml/min, using Column C18,indicating the Endosulfan content in formulation no H3.(0.025ppm), and H4,(0.04 ppm), but the pesticide contamination indicated in such herbal formulation was in permissible limit as per WHO specification(0.05 ppm).The data indicated suggest that there is requirement of in process improvement to provide better quality for consumer health in order to be competitive in international markets.

Keywords: Triphala Churna, Endosulfan content, RPHPLC,

INTRODUCTION

Pesticide residue refers to pesticides, their poisonous metabolic and degradation products and impurities that may remain on or in the organism, agricultural product, and the environment, after they are applied. Sometimes pesticide residue is unavoidable; however, if the residue exceeds the maximum limit or a tolerance, it will pose significant risk to humans and animals or other creatures in the ecological system, through the food chain.[1]

Pesticide residue – WHO and FAO (Food and Agricultural Organization) set limits of pesticides, which are usually present in the herbs. These pesticides are mixed with the herbs during the time of cultivation. Mainly pesticides like DDT, BHC, toxaphene, aldrin, and endosulfan cause serious side-effects in human beings if the crude drugs are mixed with these agents[2].

Considering the growing interest in Western countries for herbal products of Chinese origin, it was decided to perform a set of purity assays on ten Chinese crude herbal drugs chosen among the most used in Italy. Samples were screened for contamination by foreign matter, inorganic residues, heavy metals and micro-organisms.[3]

The Medicinal plants materials are liable to contain pesticide residue, which accumulate from agriculture practices such as spraying treatment of soils during cultivation and administration of fumigants during storage. It is therefore recommended that every country producing medicinal plant material should determine the pesticide residue. The organophosphorous pesticide e.g**.** parathion, Malathion and diazinon are potent cholinesterase inhibitors and can be very toxic.CNS symptoms include restlessness as well as depression of respiratory or cardiovascular system. Repeated exposure may have a cumulative action on human being. Data from animal and human exposure indicated a number of examples of chronic toxicity including carcinogenicity. W.H.O. has established the maximum residue limit (MRL) in medicinal plants. The presence of organochlorine and organophosphorous in herbal formulation is determined by oxygen combustion or gas chromatography.[4]

The WHO has also established maximum residue limit (MRL) for these cultivated or wild medicinal plants as well as appropriate methodologies for their analysis. The MRL is calculated after safety tests in human beings, which indicate toxicologically acceptable levels according to the most reliable assays available at the time. Many problems may occur in establishing the tolerable contaminant limits in products such as phytopharmaceutical Besides considering bioaccumulation, bioreactivity and synergic interactions in the toxicological assessment of a specific pesticide.The non-observable effect level (NOEL), which is defined as the highest dose (mg pesticide/kg body weight/day) that produces no observable toxic effects in the most sensitive species, is derived from chronic toxicity tests and is used to set the acceptable daily intake

(ADI) for humans: ADI. = NOEL x .Safety factor .1=100 to 1=2000.

The ADI, described as the daily intake of a chemical over a lifetime that causes no appreciable risks to human health according to our present toxicological knowledge, includes a variable safety factor, e.g. 1/100, applied in uncomplicated cases where all the required toxicological data are available.

The MRL can be calculated by using the following formula:

MRL. = ADI x W

MDI x .100 x .safety factor..

Where MRL is the maximum residue limits (mg/kg),

ADI is the acceptable daily intake (mg compound/kg body weight),

1.*W* is the body weight (kg),

2.MDI the mean daily intake of drug (kg) [5]

The organ phosphorus pesticides, e.g. parathion, Malathion and diazinon, are potent cholinesterase inhibitors and can be very toxic. CNS symptoms include restlessness as well as depression of the respiratory or cardiovascular system. Repeated exposure may have a cumulative effect although these pesticides are, in contrast to organ chlorines, rapidly metabolized and excreted and are not appreciably stored in body tissues. Among some of the other groups cited, the carbamates (N-substituted esters of carbamic acid) are cholinesterase inhibitors. They differ from the organophosphorous pesticides because their inhibitory effect is generally less intense and more rapidly reversed. Furthermore, they do not seem to enter the CNS as readily, so severe central effects are uncommon.[6]

Good Manufacturing Practice (GMP) for producing these medicinal products from herbal or natural sources. The public's belief that herbal and natural products are safer than synthetic medicines can only be ascertained by imposing regulatory standards on these products that should be manufactured using this Good Practices.[7]

About Triphala churna

Triphala is one of the well known powdered preparations of Indian system of medicine being used in Ayurveda since ancient time. This is well known phytomedicines is made in combination with Terminalia chebula, Terminalia belerica and Embilica officinalis in equal proportion as reported in Ayurvedic Formulary of India

(AFI).This formulation is prescribed in first line treatment of many ailments as Laxative, detoxifying agent and rejunevator in Ayurveda. Its anti-diabetics, anti-mutagenic, purgative and radioprotective activities have been reported.The individual herbs are reported to have several other health benefits. The Embilica officinalis is reported to possess anti inflammatory, antimutagenic, antioxidants, cytoprotective, gastro protective, and hypolipidemic activity. Similarly Terminalia possesses antibacterial, anticancer, anticaries, antimutagenic potential and inhibits local anaphylaxis. Terminalia belerica reported to possess the myocardial necrosis, reduce cholesterol induced atherosclerosis and act as hepatoprotective.[8]

Sample collection

The ten herbal formulation of Triphala churna marketed by various herbal manufacturers were collected from the retail medical stores of Yavatmal (Vidarbha region, India).

Experimental

Study design

An experimental method of research was performed to assess the presence or absence of toxic pesticide residue (Endosulfan) in selected Herbal formulations; and the concentration of each pesticide residue was determined by HPLC method.

Collection of standard sample

The standard sample of Endosulfan was procured from local market

Application of reported method on standard sample of Endosulfan[9]

The method reported was used for analysis of pesticide content in the present work for determination of Endosulfan content in Herbal formulations in which following chromatographic conditions were given

Chromatographic conditions

Mobile Phase: - 0.1% acetic acid in Acetonitrile

Flow rate: -1 ml/min.

Column: - C18

Column temp :-Ambient

Injection Volume:-10 µL

Preparation of standard

The stock solution of Endosulfan was prepared 1ml solution of Endosulfan further dilutions were made to get standard Endosulfan solution having concentration of 5µg/ml.The 20µl solution was injected at detection wavelength of 212nm by HPLC method.

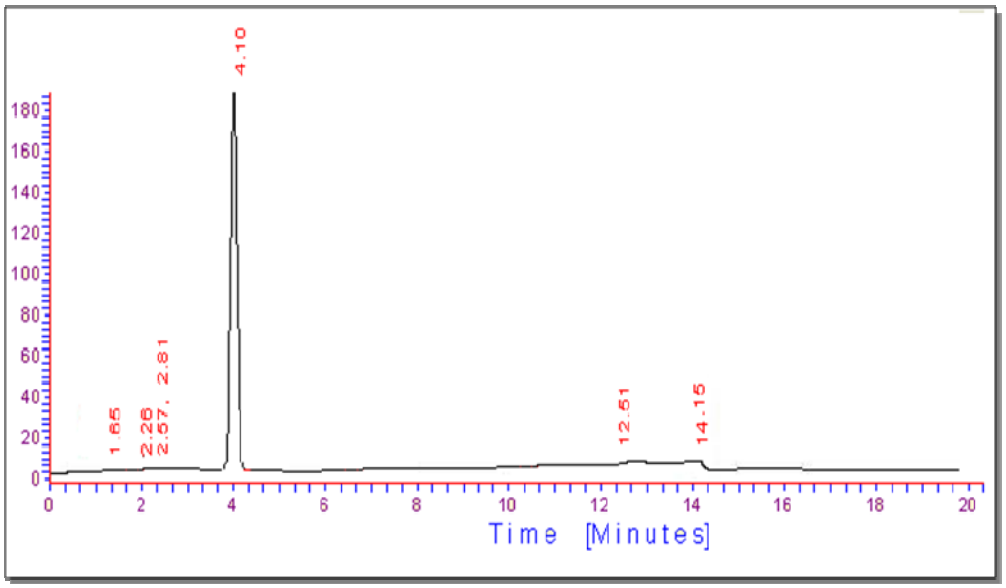

Fig. 1: HPLC chromatogram of standard Endosulfan RT 4.10

Study of the linearity range

An aliquot portion of stock solution of endosulfan was further diluted appropriately to get series of concentration in between the range of 10-100 µg/ml.The following Chromatographic conditions were established for separation of drug from all its degradation products and maintained throughout the method.

System: Thermo Separation Product Quaternary Gradient

Column: Column Machinery .Nagel (M N) EC 250/4.6 .

Stationary phase: C_{18} Nucleosil 100-5

Detector: TSP Ltd. Model number D4000

Mobile phase: Acetonitrile: acetic acid 99.9: 0.10

Detection wavelength: 212 nm

Mode: Isocratic.

Sample size : 20 µL

Temperature: Room temperature (RT)

Preparation of mobile phase

Acetonitrile was mixed in the ratio 99.90:0.10 with acetic acid. The mobile phase was sonicated for 30 min and filtered through 0.45µ membrane filter.

Procedure

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. Then each dilutions of drug were injected and peak areas were recorded. The graph is plotted as concentration of endosulfan verses area under curve.

Table 1: Concentration and peak area of Endosulfan

Sr. No.	Endosulfan (PPM)	Peak area
1	0.01	351.20
2	0.02	702.19
3	0.03	953.28
4	0.04	1403.39
5	0.05	1752.49
6	0.06	2103.58
7	0.07	2458.67
8	0.08	2809.78
9	0.09	3160.88
10	0.10	3504.78

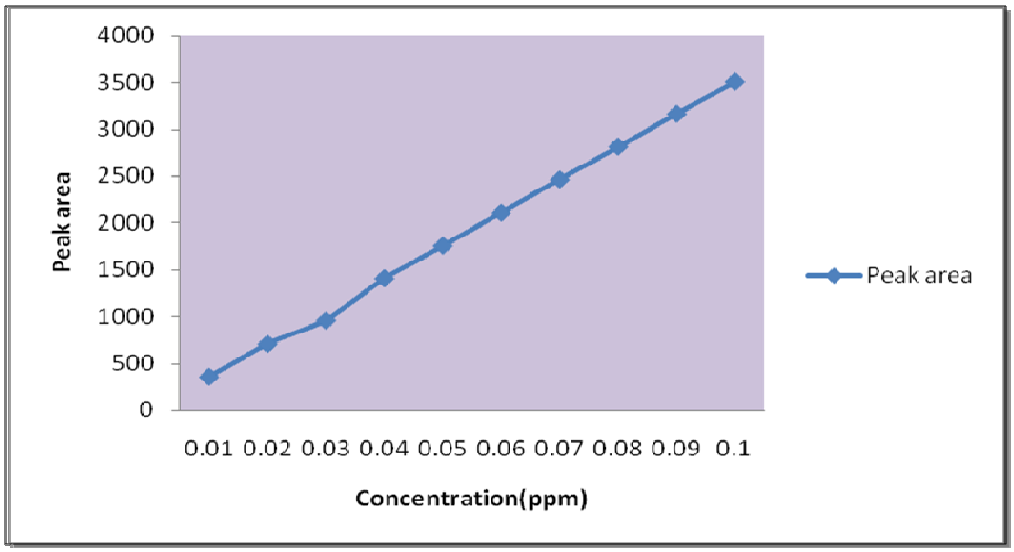

Fig. 2: Standard calibration curve of Endosulfan

Extraction of common pesticide from material

Ten grammes of the sample was taken in an R.B. flask and added sodium sulphide with 100ml n-Hexane. It was refluxed for 1 hour. The filtrate was taken in a separating funnel and extracted with 50ml and 25ml of acetonitrile.

The acetonitrile layer was mixed with 500ml DM water with 2.5ml saturated sodium sulphide and again shaken in a separating funnel with an N-Hexane layer and evaporated on a water bath. Now this residue was dissolved in acetonitrile and was used for the analysis of organochlororine.[10]

Assay of herbal formulation for quantitative determination of Endosulfan

As the herbal formulation no H3 and H4 were showing presence of Endosulfan hence assay of the two formulations H3 and H4 were carried out as follows. (WHO Permissible limit for Endosulfan in Herbal Products= 0.05 ppm).

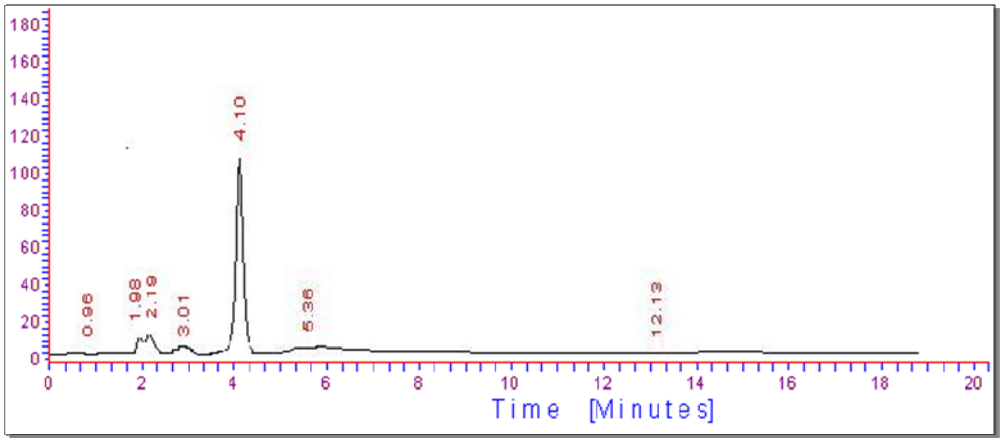

Fig. 3: HPLC Chromatogram of Endosulfan in formulation H3

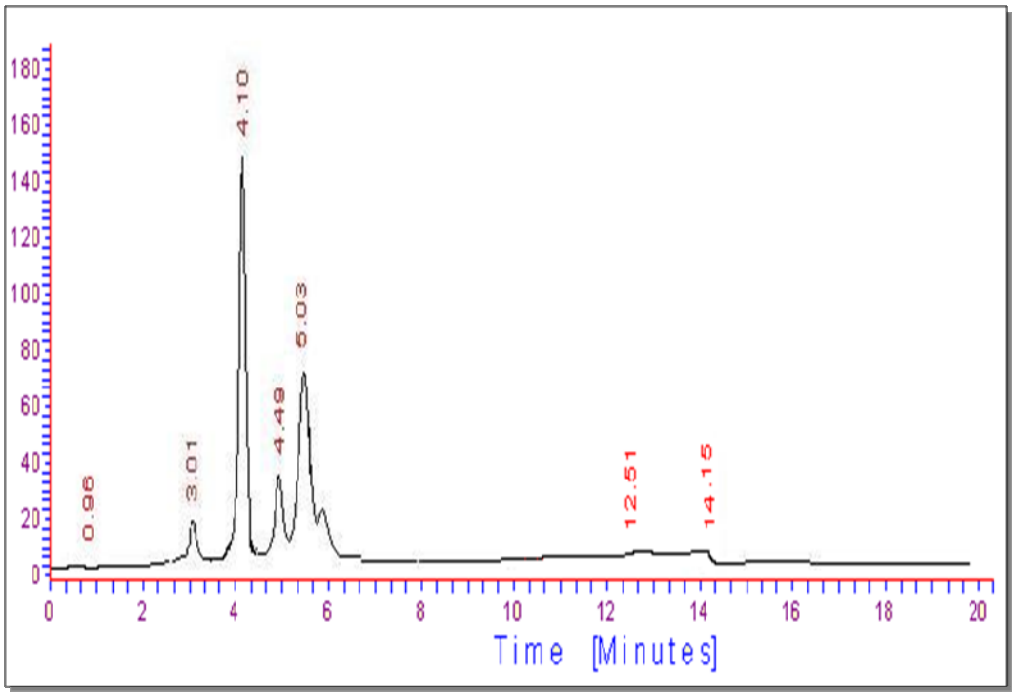

Fig. 4: HPLC Chromatogram of Endosulfan in Formulation H4

Table 2: Analysis of Herbal formulation H3 by proposed method

Sr No	Weight of Triphala powder taken (gm)	Standard peak area	Sample peak area	Amount of Endosulfan estimated in sample (ppm)	Within permissible limit or not
1	10	1751.00	879.00	0.025	Yes
2	10		876.00	0.025	Yes
3	10		875.00	0.024	Yes
4	10		876.00	0.025	Yes
5	10		876.00	0.025	Yes

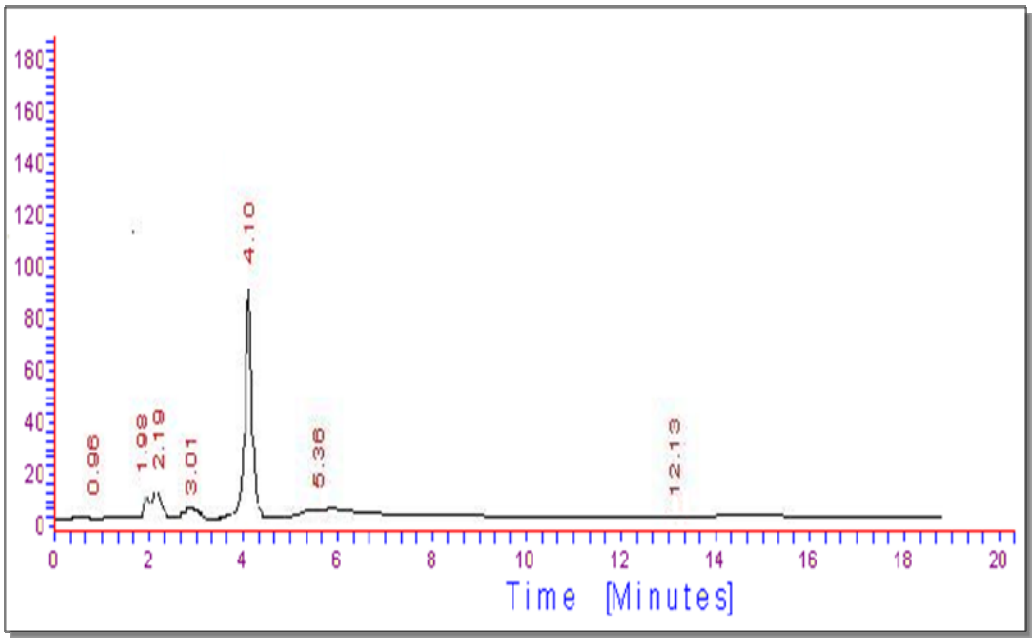

Fig. 5: Chromatogram of assay of Herbal formulation H3

Table 3: Analysis of Herbal formulation no H4 by proposed method

Sr No	Weight of Triphala powder taken (gm)	Standard peak area	Sample peak area	Amount of Endosulfan estimated in sample (ppm)	Within permissible limit or not
1	10	1751.00	1410.00	0.04	Yes
2	10		1411.00	0.04	Yes
3	10		1410.00	0.04	Yes
4	10		1417.00	0.04	Yes
5	10		1408.00	0.04	Yes

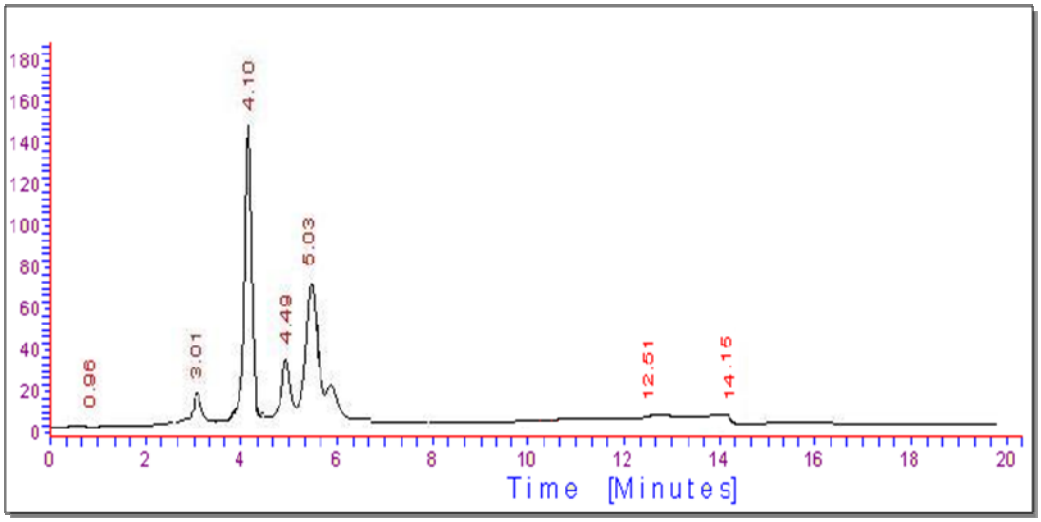

Fig. 6: Chromatogram of assay of Herbal formulation H4

Table 4: Endosulfan content in Herbal formulation code H1 to H10

Formulation code	Endosulfan content (PPM)	Remark
H1	-	Not detected
H2	-	Not detected
H3	0.025	Within permissible limit
H4	0.04	Within permissible limit
H5	--	Not detected
H6	-.	Not detected
H7	--	Not detected
H8	--	Not detected
H9	--	Not detected
H10	--	Not detected

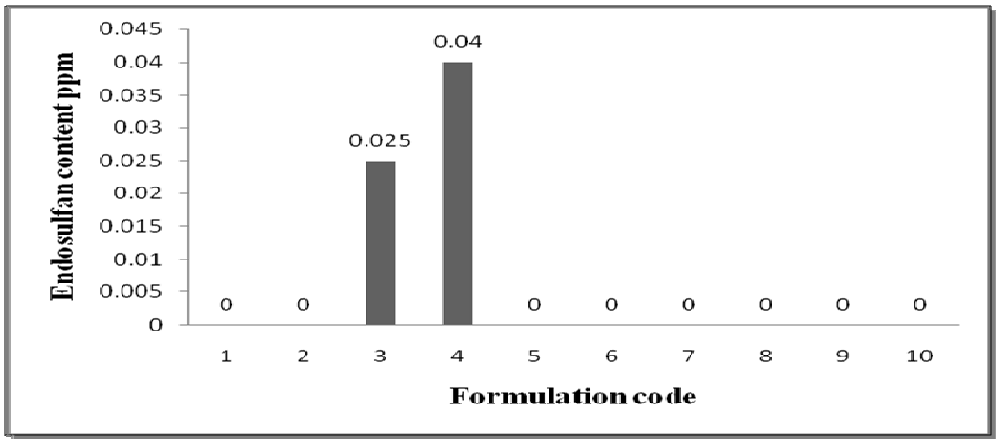

Fig. 7 : Graphical representation of Endosulfan in Herbal formulation code H1 to H10

RESULTS AND DISCUSSION

WHO prescribed maximum residual limit for pesticide contamination. The present work indicate that the pesticide residue content is shown in formulation no H3.(0.025ppm), and H4,(0.04 ppm),the presence of pesticide residue can produce serious adverse effect on body of the patient using this medicine for longer duration, but the pesticide contamination indicated in such herbal formulation is in permissible limit as per WHO specification(0.05 ppm)

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