Research Article

Standardization and Evaluation of some Parameters of Adaptogenic Polyherbal Oral Dosage Form

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ABSTRACT

In present study the Polyherbal capsule formulation containing extracts & powder of various plant constituents i.e. *Withania somnifera, Chlorophytum tuberosum, Mucuna puriens, Tribulus terrestris, Blepheris edulis, Puraria tuberosa, Myristica fragrance, Hygrophila spinosa, Piper cubeba,* and *Cinnamomum camphor, Syzygium aromatocum, Zingiber officinalis, Piper nigrum, Piper longum, , Crocus sativus,* excipients. The drugs were selected on basis of their synergistic action for adaptogenic activity. Standardization protocol included different parameters such as estimation of active constituents in formulation by quantitative methods, Heavy metal analysis, and TLC Profile were also done. Formulation also evaluated for acute toxicity study. The data generated from the present study would help in the authentification and produce standardized formulation. Treatment with formulation gives significant results. The results of the acute toxicity test, for oral preparation of capsule formulation indicate that it is relatively safe and non-toxic to rats. The findings of these experimental produce the standardized capsule formulation and acute toxicity indicate that capsule formulation possesses potential adaptogenic activity.

Keywords: Polyherbal capsule formulation, Adaptogenic activity, Acute toxicity study, Standardization, Heavy metal.

INTRODUCTION

WHO has defined herbal medicines as finished labeled medicinal product that contains active ingredients, aerial or underground parts of the plant or other plant material or combinations[1].

Polyherbal capsule formulation containing extracts and powder of various plant constituents i.e. *Withania somnifera* (Ashwagandha), *Chlorophytum tuberosum* (Swet musali), *Mucuna puriens* (Kaunch), *Tribulus terrestris*(Gokharu), *Blepheris edulis*(Uttingan), *Puraria tuberose* (Vidarikand), *Myristica fragrance*(Jathiphal) , *Hygrophila spinosa* (Talamkhana), *Piper cubeba* (Kankol), and powder of *Cinnamomum camphor*(Karpoor), *Syzygium aromatocum*(Lavang), *Zingiber officinalis* (Sunth), *Piper nigrum* (Kali mirch), *Piper longum* (Pippali), *Crocus sativus*(Kesar) excipients. Tablet was formulated at Vasu Healthcare Pvt. Ltd., Vadodara, India. All the extracts and powder were vacuum dried.

Plants and its part such as *Withania somnifera* (Root), *Chlorophytum tuberosum* (Tuber root), *Mucuna puriens* (Seed), *Tribulus terrestris*(Fruit), *Blepheris edulis*(Seed), *Puraria tuberose* (Tuber), *Myristica fragrance*(Seed) , *Hygrophila spinosa* (Seed), *Piper cubeba* (Fruit), and powder of *Cinnamomum camphor*(Satva), *Syzygium aromatocum*(Flower bud), *Zingiber officinalis* (Rhizome), *Piper nigrum* (Fruit), *Piper longum* (fruit), *Crocus sativus*(Satva) were procured from Vasu Healthcare Pvt. Ltd., Vadodara, India, for the microscopy. All these plants were identified and authenticated by Mrs Darshika Nayan Shah (Ph.D.) (Botany)

All the reagents and instruments used for standardization and evaluation of adaptogenic Polyherbal oral dosage form was facilitated by Vasu Research Centre - A division of Vasu Healthcare Pvt. Ltd., Vadodara.

MATERIALS AND METHODS

Collection and Authentification of Raw material and Finished Product

Tablet was formulated at Vasu Healthcare Pvt. Ltd., Vadodara, India. All the extracts and powder were vacuum dried. Plants and its part were procured from Vasu Healthcare Pvt. Ltd., Vadodara, India, for the microscopy. All these plants were identified and authenticated by MrsDarshika Shah (Ph.D.) (Botany). All the reagents and instruments used for standardization and evaluation of adaptogenic Polyherbal oral dosage form was facilitated by Vasu Research Centre - A division of Vasu Healthcare Pvt. Ltd., Vadodara.

Assays for individual components

Total alkaloids by gravimetric method[2]

3 gm of sample or test substance and transferred into 150ml of conical flask. 5ml of ammonia solution was added. Frequently shacked for 5 min. 100ml of mixture of solvent ether: methanol (80:20) was added for an hour with shaking. Decanted the filter through cotton plug and clear solution taken into a separator and the residue washed with 100ml mixture of ether: methanol (80:20) (20×5). To the total ether: methanol solution 30ml of 1N H2SO4 was added. Well shacked and allowed to separate. Lower layer collected. The extraction was continued with above upper layer with 25ml and then with successive

quantity each of 25ml of a mixture of 0.5N H_2SO_4: methanol (75:25) until complete extraction of the alkaloid was affected. The mixed acid solution was washed first with 10ml chloroform and then with two successive quantity of chloroform (2×5). Each chloroform extract was washed (i.e. lower layer) with same 20ml of acid: methanol mixture (15: 5). The chloroform layer was rejected and transferred the acidic liquid into the conical flask which contains acidic solution. Made it alkaline with dil. Ammonia solution and added 5 ml excess. Shake first with 25ml then with successive quantity each of 20 ml of chloroform until complete extraction of the alkaloid is affected. Washed each chloroform with 10ml water. Evaporated chloroform layer on water bath and 2ml of methanol was added and continued evaporation on a water bath till dryness. Continue drying at 80 C in oven till constant weight.

$$\text{Total alkaloid (\%)} = \frac{\text{Weight of residue} \times 100}{\text{Weight of substance taken}}$$

Saponins by gravimetric method

5gm of powder were accurately weighed in weighing dish. Then Transfer it to a clean, dry ground glass stopper conical flask. To that 50ml of 90%v/v methanol was added. Mixed well and reflux for ½ hr. then Cool and filtered. Residue washed if any with 90% v/v methanol till washings are almost colorless. Combined the methanol extract. Evaporated on water bath to obtain a thick paste like residue. Residue was treated with 25ml petroleum ether. Separated the petroleum ether layer and discard it. Then again treat this residue with 25ml chloroform. Separated the chloroform layer and discard it. Treat that chloroform wash residue with 25ml of ethyl acetate. The ethyl acetate layer was separated and discards. To that ethyl acetate washed residue 5ml of 90% v/v methanol was added. Then pour that drop wise with constant stirring into a beaker containing 25ml acetone to obtain precipitate. The flask containing residue was rinsed with minimum volume (about 2ml) of 90% v/v methanol. Transferred it to same beaker for precipitation with constant stirring. Than organic layer was decanted and dry the precipitate to the constant weight at 100 C in oven. Calculated % of total Saponins using the formula given below.

$$\text{Total Saponins (\%)} = \frac{\text{Weight of residue} \times 100}{\text{Weight of sample taken}}$$

***L-DOPA*[3]**

Part A: accurately weighed 0.1gm of *Mucuna puriens* extract in 250ml conical flask. Dissolved in 10ml of formic acid. 80ml of glacial acetic acid and solvent blue-19 was added (oracet blue B) as indicator.

Part B: 0.1gm of mercury acetate was added in 250ml conical flask. 10ml glacial acetic acid was added to dissolve it properly and mixed well.

Titration

Transferred part B into part A. Immediately titrate mixture against 0.1N perchloric acid. Note down the burette reading.

Blank determination

Blank determination was performed same as described above but without the extract material.

Burette reading: burette reading – blank reading

$$\text{L-DOPA (\%)} = \frac{\text{Burette reading} \times 0.01972 \times \text{N of } HClO_4 \times 100}{0.1 \times \text{weight of extract in gm}}$$

Flavones

1gm of sample was added to 60ml alcohol. Shacked for 1 hr. put it for 1 hr. filtered it. Washed with alcohol twice (50×2). Then Combine filtrate and wash with alcohol and concentrated it up to 10ml. 10ml concentrated solution add drop wise with constant stirring to 100ml ether. Keep for 10min for settling. Filtered and evaporated filtrate to dryness. Dried it to constant weight.

$$\text{Flavones (\%)} = \frac{c \times 100}{y}$$

Where;
y = weight of sample taken
a = weight of beaker
b = weight of beaker + residue
c = weight of residue (b-a)

Mucilage

Weighed accurately 1 gm of sample in conical flask and 100ml of water added. Shake well for 2 hrs. Keep for overnight than filtered it and concentrated to 10 ml. Pourthe solution to 50ml of 90% alcohol with continuous stirring. Filtered through a weighted filer paper and collected the precipitate. Dry it to constant weight and weigh it and calculate the % of mucilage.

$$\text{Mucilage (\%)} = \frac{d \times 100}{a}$$

Where;
a = weight of sample
b = weight of filter paper
c = weight of filter paper + residue
d = weight of residue

Piperine

Standard preparation

Weighed 0.02gm pure piperine. Dissolved in 30ml methanol on water bath. Filtered in volumetric flask (100ml). Make up the volume with methanol. Pipette out 5ml in 50ml volumetric flask. Make up the volume with methanol. Measured the absorbance at 342nm.

Sample preparation

Weighed 0.5gm extract. Dissolved in 30ml methanol on water bath. Filtered it in 100ml volumetric flask. Make up volume with methanol. Pipette out 5ml in 50ml

volumetric flask. Make up the volume with methanol. Measured the absorbance at 342nm.

$$\text{Piperine (\%)} = \frac{T \times Cs \times P \times 100 \times 100}{S \times Ct \times (100 - LOD)}$$

Evaluation of Quality Control Parameters for Capsule

Description[4]

The general appearance, its visual identity is essential for consumer acceptance, for control of lot-to-lot uniformity and for monitoring trouble free manufacturing. The control of the general appearance of tablet and capsule involves the measurement of a number of attributes such as size, shape, color, etc.

Uniformity of weight for capsule[5, 6]

Intact capsule selected at random were weighed. Open the capsule without losing any part of the shell and remove the contents as completely as possible. The shell was weighed. The weight of the contents was the difference between the weighing. The procedure was repeated with a further 19 capsules. Not more than two of the individual weights deviatefrom the average weight by more than the 5 percentage in case of average weight of capsule 250 or more and also none of deviate by more than twice that percentage.

Disintegration test for capsule[5, 7]

This test was performed to check whether capsules disintegrate within a prescribed time when placed in a liquid medium under the prescribed experimental condition. Disintegration test was performed using the digital microprocessor based disintegration test apparatus by VEEGO.

One capsule was introduced into each tube and added a disc to each tube. The assembly was suspended in the water in a 1000 ml beaker. The volume of water was such that the wire mesh at its highest point is at least 25 mm below the surface of the water, and at its lower point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained the temperature at 37o C ± 2o C. Noted down the time require to all capsules disintegrate and pass through wire mesh.

Dissolution test for capsule[6, 7]

Dissolution test was performed to check the how much amount of drug dissolves in specific medium in specific time. This dissolution test was performed for capsule using USP dissolution apparatus 2 by VEEGO. The 900 ml of the 5N HCl as dissolution medium was introduced into the vessel of the apparatus. The dissolution medium was warmed to 36.5OC- 37 OC for the capsules basket type dissolution apparatus were used. The apparatus was operated immediately at the speed of 50 rpm for two hrs. After two hrs a 25 ml specimen was withdrawn from a zone midway between the surface of the dissolution medium and top of the rotating blade or basket, in evaporating dish. Filter and evaporate the specimen. For the each of capsule tested, the amount of dissolved active ingredient in the solution was calculated as a percentage dissolved in two hrs.

Determination of Moisture content[8]

Karl Fischer titration method was carried out by using Karl Fischer apparatus. In the apparatus, sufficient quantity of methanol was added to dip the electrodes in thebeaker and the methanol was neutralized with Karl Fischer Reagent.75mg of distilled water was added and the addition of Karl Fischer Reagent was started till end point was reached to calculate the factor

$$\text{Karl Fischer factor} = \frac{\text{Weight of water}}{\text{Volume of Karl Fischer Reagent}}$$

Then, approximately 0.1 gm of each sample was added in the beaker and started stirring. Karl Fischer Reagent was added automatically till the end point was reached. The reading was noted down that was displayed on the screen and the % of moisture was calculated by the given formula

$$\text{\% of moisture content} = \frac{\text{Factor x reading x 100}}{\text{Weight of the sample}}$$

TLC finger-printing of raw materials and finished product

Preparation of reference solution (S1)

1.0g of extract (Reference Standard) was taken with 25 ml of Methanol & refluxed it on water bath at 90-1000C for 15 min. Filtered and evaporated up to 5ml in porcelain dish. Take the solution was taken for TLC Profiling**.**

Preparation of test solution (T1)

1.0g of sample extract (Test Material) was taken with 25 ml of Methanol & reflux it on water bath at 90-1000C for 15 min. Filtered and evaporated up to 5ml in porcelain dish. Take the solution for TLC Profiling.

Preparation of Spray reagent [Anisaldehyde – sulphuric acid reagent]

0.5 ml Anisaldehyde EP is mixed with 10 ml Glacial acetic acid AR, followed by 85 ml Methanol AR and 5 ml Sulphuric acid 98% GR. Solvent Systems for the raw materials were decided.

Heavy Metal Analysis

Preparation of test solution[8]

Sample preparation (Applicable for Finished product and raw material)

Accurately weighed 0.5g or 0.5mL sample in PFA (Perfluoroalkoxy) Teflon vessels. 8mL 69% Nitric acid (HNO3) was added along the sides of the vessel so as to remove the adhering matter. If the effervescence was formed, then let vessel open for 30 minutes in fumigation chamber. Thevessels was closely tightly and keep on the turner. Set the method parameters was as follows:

Table 1:

Vessel no.	Power		Ramp (Min)	°C (control)	Hold time (min)
	MAX	%			
8	800 W	100	20	180	10
16	1600 W	80	20	180	10

After digestion procedure was complete. The vessels were taken out and kept in fumigation chamber for 10 minute. The vessels were slowly opened to release the pressure from the vessel. Leave the vessel undisturbed till the fumes were released competently. The solution was transferred into 50ml volumetric flask using funnel. The volume was make up with distilled water. Shake welled and filtered using whatman filter paper No.1. Used this solution as a test solution. Prepared synchronously the blank solution.

Sample preparation (Applicable for Inorganic materials)

Accurately weighed 0.25g sample in PFA (Per fluoro alkoxy) Teflon vessels. 4.8ml69% Nitric acid (HNO3) and 1.2ml conc. HCl was added along the sides of the vessel so as to remove the adhering matter. If the effervescence is formed, then let vessel open for 30 minutes in fumigation chamber. Closed the vessels tightly and keep on the turner. Set the method parameters were as follows:

Table 2:

Vessel no.	Power		Ramp (Min)	°C (control)	Hold time (min)
	MAX	%			
8	800 W	80	30	180	10
16	1600 W	80	30	180	10

After digestion procedure was completed. The vessels was taken out and kept it in fumigation chamber for 10 minute. The vessels were opened slowly to release the pressure from the vessel. Leave the vessel undisturbed till the fumes were released competently. Again 3ml conc. Hydrochloric acid (HCl), 0.5ml Hydrofluoric acid (HF), 0.5ml Hydrogen peroxide (H2O2) was added in the vessel. Kept it for pre-digestion (till effervescence stop). Closed the vessels tightly and kept for the digestion by setting method parameters as above. After digestion procedure is completed. The vessels were taken out and kept it in fumigation chamber for 10 minute. Opened the vessels slowly to release the pressure from the vessel. Leave the vessel undisturbed till the fumes were released competently. Transfer the solution in 50ml volumetric flask using funnel. Make up the volume with distilled water. Shake well and filtered using whatman filter paper No.1.Use this solution as a test solution. Prepare synchronously the blank solution.

Detection

Then samples were analyzed for the presence of Pb, Cd, as and Hg using Atomic absorbance spectrophotometer (AAS) 6300 (by SHIMADZU)

Test for toxicity study[9]

Group	Treatment	Dose (mg/kg/day)	Number of animals	Route of Administration
I	Normal (Saline)	Vehicle	6	Oral
II	Group 1	200 mg/kg,	6	Oral
III	Group 2	500 mg/kg,	6	Oral

Methodology: 1. For FST & TST all ten group treatment will be selected and for CMS & LMA first four group treatment will be given. Total duration of treatment for FST, TST is 7 days and for CMS it is 21 days. LMA will be performed for two days.
2. FST-Forced swim test, TST-Tail suspension test, CMS-Chronic mild stress test. LMA –Locomotor activity.
Clinical observations: N/A
Termination: In case of FST, TST, at the end of 7th day behavioural test will be performed.In case CMC following 21 days stress treatment, animal will be subjected to weekly test for 21days.Locomotor activity will be measured after test dose.

Table 3:

	FST	TST	CMS	LMA
Parameters to be evaluated	Immobility, swimming and climbing time	Immobility time	Sucrose intake	Spontaneous motor activity
Statistical Analysis	By suitable Statistical methods			

RESULTS AND DISCUSSION

Assay for individual compound present in raw materials

Assay of all the individual ingredients of “capsule” given in the Table no. 4.

Results shown the active principle present in the extract by assay, this indicate that the active constituents of all the extracts follows the desirable specification of extracts & powder.

Table 4: Assay of all the individual ingredients of 'capsule'

Sr. no.	Name of ingredients	Assay for	Result %(w/w)
1	*Withania somnifera* (Ashwagandha)	Alkaloids	0.33%
2	*Chlorophytum tuberosum* (Swet musali)	Saponin	36.396%
		Mucilage	13.16%
3	*Mucuna puriens* (Kaunch)	L-Dopa	47.328%
4	*Tribulus terrestris* (Gokharu)	Saponin	10.8%
5	*Blepheris edulis* (Uttingan)	Saponin	5.70%
6	*Puraria tuberose* (Vidarikand)	Flavones	10.144%
7	*Myristica fragrance* (Jathiphal)	Resin	5.2%
8	*Hygrophila spinosa* (Talamkhana)	Mucilage	13.8%
9	*Piper cubeba* (Kankol)	Piperine	51.720%
10	*Piper nigrum* (Mirch)	Piperine	36.11%
11	*Piper longum* (Pippali)	Piperine	53.1307%

Quality Control Parameters for 'capsule'

Quality control parameters like description, Uniformity of weight, pH, disintegration, dissolution, moisture content and powder characteristics for different batches of "capsule" are given in Table 5.

Table 5: Quality control parameters of 'capsule'

Parameter	Batch no.	Batch no.	Batch no.
Description		**Hard gelatin capsule**	
Cap Body Size	Golden Golden "0"	Golden Golden "0"	Golden Golden "0"
Physicochemical parameters			
Uniformity of weight	521.90±mg	520.89	521.78
pH	5.41	5.34	5.21
Disintegration	5min 31 sec	5min 30 sec.	5min 31 sec
Dissolution	73.53%	72.90%	73.89%
Moisture content	8.28%	8.32%	8.30%
Powder characteristics			
Bulk density	0.5	0.5	0.5
Tapped density	0.62	0.62	0.62
Hausner‟s ratio	1.24	1.24	1.24
Carr's index	19.35	19.35	19.35

All the batches had uniformity in color of cap was black and body was amnesty and zero size capsule. Uniformity of weight of capsules of each batches were almost same. The capsules were disintegrated within 5 min 31 sec. and had about 73.53% w/w dissolution. Moisture content in all the batches was 8.28 % w/w and pH was 5.41 indicating slightly acidic. Hausner‟s ratio was 1.24 indicates powder having good flowing property. Carr's index was 19.35 indicates powder having good compressibility. Result indicated that all three batches of „capsule" were uniform in colour, size, weight, dissolution and disintegration time, moisture content, angle of repose, bulk and tap density etc.

Estimation and Fingerprinting of Finished Product by TLC method

Table 6: TLC fingerprinting of finished product

Finished product		
Sr. No.	**Comparative Rf value**	**Colour of band**
1	0.75	Green
2	0.66	Violet
3	0.53	Green
4	0.46	Pink
5	0.26	Light violet

Heavy Metal Analysis

Analysis of Heavy metal like Lead (Pb), Cadmium (Cd), Mercury (Hg), Arsenic (As) were carried out using Atomic absorbance spectrophotometer (6300) by Results are shown in Table 7.

Results indicate that concentration of lead, cadmium, mercury and arsenic in extract, powder and capsule were not more than limit. So this indicates that extracts, powder and capsule pass the limit for heavy metal.

Acute Toxicity Study

In the preliminary acute toxicity study, capsule seems to be safe at 2000 mg/kg and 5000 mg/kg higher dose.

Table 7: Heavy Metal Analysis of 'capsule'

Sr.No.	Material Name	Mercury (Hg) (NMT 1 ppm)	Lead (Pb) (NMT 10 ppm)	Cadmium (Cd) (NMT 0.3 ppm)	Arsenic (As) (NMT 3 ppm)
1	*Withania somnifera (Ashwagandha)*	Absent	0.112	Absent	0.078
2	*Chlorophytum tuberosum* (Swet musali)	Absent	0.042	Absent	0.124
3	*Mucuna puriens* (Kaunch)	Absent	Absent	Absent	Absent
4	*Tribulus terrestris* (Gokharu)	Absent	Absent	Absent	Absent
5	*Blepheris edulis* (Uttingan)	Absent	Absent	Absent	0.043
6	*Puraria tuberose* (Vidarikand)	Absent	Absent	Absent	Absent
7	*Myristica fragrance* (Jathiphal)	Absent	0.326	Absent	0.102
8	*Hygrophila spinosa* (Talamkhana)	Absent	Absent	Absent	Absent
9	*piper cubeba* (Kankol)	Absent	Absent	Absent	Absent
	Powder of:				
10	*Syzygium aromatocum* (Lavang)	Absent	0.124	Absent	0.047
11	*Zingiber officinalis* (Sunth)	Absent	Absent	Absent	Absent
12	*Piper nigrum* (mirch)	Absent	0.1164	Absent	Absent
13	*Piper longum (pippali)*	Absent	0.142	Absent	0.126
	Finished product:				
14	Spark royal cap.	Absent	0.747	Absent	0.345

There was no toxic or deleterious effects were observed immediately in 24 hours or during 48 hours and up to 14 days of observation period. There was no major change in body weight and no mortality found in any animal.

Table 8(A): Cage side observations of animals

S.No.	Parameters	Capsuleobservation	
		2000 mg/kg	5000 mg/kg
1	Condition of fur	Normal	Normal
2	Skin	Normal	Normal
3	Subcutaneous swelling	Nil	Nil
4	Eyes dullness	Nil	Nil
5	Eyes opacities	Nil	Nil
6	Colour and consistency of faeces	Normal	Normal
7	Condition of teeth	Normal	Normal
8	Breathing abnormalities	Nil	Nil

Table 8(B): Mortality Record of Capsule

Group	Dose (mg/kg body wt.)	Mortality	
		Male	Female
I	2000	0/3	0/3
II	5000	0/3	0/3

SUMMARY AND CONCLUSION

Standardization

All data from results suggested that capsule and its composition were consistent with various quality and purity parameters.

Acute toxicity study

The result of the acute toxicity test, for oral preparation of capsule formulation indicated that it is relatively safe and non-toxic to rats.

Table 8(C): Mean Body weight & percentage body weight gain in Capsule

Group	Dose (mg/kg body wt.)	Body weight		% body wt. gain day 1-7	Body weight	% body wt. gain day 7-14	% body wt. gain day 1-14
		Day 1	Day 7				
Control	-	238.62	249.6	4.62 %	255.63	10.18%	19.93%
I	2000	229.45	244.36	6.49 %	247.48	10.29%	20.07%
II	5000	215.6	228.06	3.08%	245.52	10.33%	20.35%

REFERENCES

1. Florey, H. W, Chain, E. and Florey, M. E, 1949. Vol. 1, The antibiotic, Oxford University Press, New York, 576-628.
2. Dr.V.Rajpal, Standardizton of botanicals- testing and extraction methods of medicinal herbs, volume 1, eastern publishers- New Delhi, 256.
3. Dr. V.Rajpal, Standardization of Botanicals (testing and extraction methods of medicinal herbs), vol.-1, Eastern publishers, New Delhi, India, 2002, pp 165-173.
4. Lachman L, Lieberman HA, Kanic JL, The Theory and Practice of Industrial Pharmacy, Third edition, Vargeshe Publishing House, Bombay, 1987, 29-303.
5. Lachman L, Lieberman HA, and Kanic JL, The Theory and Practice of Industrial Pharmacy, 3rd Edn, Vargeshe Publishing House, Bombay, 1987, 29-303.
6. Indian Pharmacopoeia, The Controller of Publication, Delhi Vol-1, 1996, 134-135.
7. Lachman L, Lieberman HA, and Kanic JL, The Theory and Practice of Industrial Pharmacy, 3rd Edn, Vargeshe Publishing House, Bombay, 1987, 29-303.
8. Lohar DR, Protocol for testing of Ayurvedic, Siddha & Unani Medicines, Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicines, Ghaziabad 2007, 47-52.
9. OECD guidelines.

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