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Pharmaceutical standardisation and analytical validation of herbal formulation capsule - UNEX

Research Article

Sarang Deshpande[1], Anita Wanjari[2], Swagata Dilip Tavhare[3*], Abhishek Patle[4]

1. PhD Scholar, 2. Professor, Mahatma Gandhi Ayurved College and Hospital Sawangi, Meghe, Wardha. India.
3. Associate professor, Department of Dravyaguna, Dr. D. Y. Patil College of Ayurved & Research Centre, D.Y. Patil Vidyapeeth (Deemed to be university), Pimpri, Pune. India.
4. Quality Control Department, Unijules Life Sciences Ltd., Kalmeshwar, Nagpur. Maharashtra. India.

Abstract

Standardisation and validation for herbal preparation using modern parameters of analytics are crucial in present era. Capsule Unex is one such formulation useful for renal diseases like urinary tract infections, renal stones, blood purifier etc. The study has been specifically performed on the latest scientific measures to ensure capsules quality standards and validate it analytically. Three batches of capsule Unex were manufactured and tested at Unijules Life Sciences Ltd. Nagpur (MS), India for organoleptic, physicochemical and physical characters, heavy metal, pesticide residue, microbial, fungal and mould limits, along with a chromatographic evaluation. All the parameters were checked against established analytical specifications and batch to batch variation was analysed. The capsule weighs of 500 mg with variation of defined limit ± 7.5 %, disintegration time (NMT 30 min), bulk density (0.71 to 0.81 g/ml), loss on drying (NMT 10 % w/w), pH (4 to 6), percentage total ash (NMT 16.3 %w/w), percentage acid insoluble ash (NMT 5.1 %w/w), percentage water soluble extractives (NLT 26.0 %w/w), percentage alcohol soluble extractives (NLT 11.6 %w/w). Fingerprinting assay revealed Rf values of *Gokshura* and *Punarnava* at 254 and 366 nm as described in API. Capsules were observed free from heavy metals like lead, arsenic, cadmium, mercury; pesticide namely organochlorine, organo-phosphorous and pyrethroids, microbes like *Enterobacteriaceae*, E. coli, *Samnonella*, *Staphylococcus*, *Psedomonas aeruginosa*, aflatoxin B1 and aflatoxin B1+B2+G1+G2 free. Total microbial, yeast and mould count was within the prescribed limits indicating good manufacturing practices. This study reveals the manufacturing of capsule Unex does complies API standards of raw material and in-house standards of capsule Unex specifications and will serve as therapeutically safe and efficacious formulation.

Keywords: Standardisation, Herbs, Healthcare, Heavy metals, Pesticides, Quality.

Introduction

A medicine whether synthetic or of herbal origin, must fulfil the minimal requirements of being safe and therapeutically effective.(1) Standardisation of herbal drugs defines the process of evaluation of the quality and purity of crude drug on various parameters like morphology, physical, chemical and biological observation.

Recently, Ayurvedic medicine is taking larger interest among researchers as on the global perspective, there is a shift towards the use herbal medicine due to reported side effects, shortcomings and dangers of some of synthetically manufactured drugs. It is the prime responsibility of the regulatory authorities to ensure that the patients or consumers get the guaranteed medicines having purity, safety, potency and efficacy. WHO guidelines states to ensure quality control of medicinal plant products by using modern technique and by applying suitable parameters and standards. (2)

Geographic condition like soil, habitat and climatic variation causes impact on phytochemical constituents of the herbs. Hence, herbal medicines are considered valid for use unless it proves the reproducibility of batch-to-batch manufacturing. Standardisation defines minimal inherent variation of composition through quality assurance.

Capsule Unex is a formulation comprises two herbs namely *Gokshura* and *Punarnava*. The present study reports pharmaceutical and analytical standardisation of Capsule Unex.

Standardisation of such Ayurvedic products can be achieved by using GMP (Good Manufacturing Practices) and GLP (Good Laboratory Practices) guidelines.

Materials and methods

The present study was successfully conducted at Unijules Life Sciences Ltd. Nagpur (MS), India; a WHO-GMP approved Ayurvedic Pharmaceutical

*** Corresponding Author:**

Swagata Dilip Tavhare

Associate professor, Department of Dravyaguna,
Dr. D. Y. Patil College of Ayurved & Research Centre,
D.Y. Patil Vidyapeeth (Deemed to be University),
Pimpri, Pune. India.
Email Id: drswagata32@gmail.com

Company. All the chemicals used in the experimentation were of analytical grade and procured from Merck Specialties Pvt. Ltd. Mumbai (India).

Capsule Unex was prepared by using herbs namely *Gokshura* (*Tribulus terrestris* L) and *Punarnava* (*Boerhavia diffusa* L). Herbal ingredients were procured from authentic sources in Nagpur region. The quality and authenticity of all the ingredients was confirmed as per the physicochemical and analytical specifications of Ayurvedic Pharmacopeia of India (API) at quality control laboratory of Unijules Life Sciences. An aqueous extract of *Gokshura* and *Punarnava* in the equal proportion was used to manufacture capsule. The capsules were prepared by wet granulation method, by coating extracts on inert bids (circular calcium inert material which acts as a adsorbent for herbal extracts) and further coated with approved colours for each extract bid and all mixed pellets are filled in a transparent capsule of size '0' (weight of empty capsule 100 mg) weighing total 470mg pellets in each capsule and total capsule weight 570mg.

Capsules were prepared in three batches by repeating the manufacturing process was termed as S-1, S-2 and S-3.

Organoleptic characters like **c**olour, odour, taste, shape were evaluated by standard procedure. (3)

Physicochemical parameters

Physicochemical parameters namely pH, loss on drying, total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractives etc. were measured as per standards of API. (4)

Heavy metal analysis

Heavy metal analysis for detection of elemental constituents in the capsule were done by using the Thermo Elemental M5 Atomic Absorption Spectrophotometer (AAS) (SPECTRO Analytical Instruments GmbH, Germany and Model: ARCOS, Simultaneous ICP spectrometer), fitted with graphite furnace and an auto sampler at lab of Unijules life science. Four heavy metals namely Cadmium (Cd), Arsenic (Ar), Mercury (Hg) and Lead (Pb), were analysed in each batch sample. An aliquot of 1 mL from three batch of samples were placed in a 250 mL beaker to which 5 mL freshly prepared acid mixture of concentrated HNO_3, concentrated HCl, and H_2O was added in the proportion of 1.5 : 0.5 : 0.5. It was further placed on hot plate and provided with gentle heat; maintaining a temperature of 150°C till the sample completely gets dissolved to form a clear solution. For prevention of sample loss during digestion process, the inner walls of the beaker were washed with deionized water. After the digestion, the samples were made up to 50 mL with deionized water and were further analysed. Multi-element standard solutions of four involved testing metals were prepared by diluting 1000 mg/L stock solutions with 5 % HNO3 solution. Samples were analysed in duplicate and the average was calculated. (5).

Pesticide analysis

The pesticide residue content for organochlorine pesticides, organo-phosphorus pesticides and pyrethroids was analysed at the Unijules Life science ltd. following a modified procedure developed in accordance with the API standard combined with LC–MS/MS analysis.

Microbial and fungal load analysis

All the three batch sample were analysed for total microbial plate count, total yeast and mould count, *enterobacteriaceae*, *E. coli* count, *salmonella* species, *staphylococcus aureus*, *psedomonas aeruginosa*, aflatoxin B1, B1+B2+G1+G2 by using nutrient agar, MacConkey agar, *Salmonella* agar, *Shigella* agar and potato dextrose agar. The microbial loads of the sample were assessed in the microbiology laboratory of Unijules life science. Samples were analysed in duplicate and the average was used.

High-performance thin-layer chromatography

Preparation of test sample: Two gram powder of *Tribulus terrestris*, *Boerhavia diffusa* and capsule Unex was accurately weighed in a conical flask to which 20 mL of methanol was added. It was refluxed for 15 min on water bath and filtered through Whatman filter paper no. 1. The filtrate is transferred in an evaporating dish for drying. The sample was reformed with 10 ml of methanol and the obtained test solution was subjected to chromatographic analysis.

Preparation of spray reagent

It was prepared by mixing anisaldehyde, glacial acetic acid, methanol and 98% sulphuric acid in the proportion of 0.5 ml, 10 ml, 85 ml and 5ml respectively.

Instrumentation and chromatographic conditions

CAMAG Linomat 5 was used for band application. An aluminium sheets pre-coated with silica gel 60 F_{254} (Merck); were developed up to 80 mm with a solvent system of toluene: ethyl acetate: formic acid, 10:3:1 v/v in CAMAG glass twin-through chamber. The chamber was previously saturated with mobile phase vapour for 30 min at 25°C. The densitometric scanning was performed at absorbance 254 and 366 nm after derivatization with anisaldehyde sulfuric acid reagent. The plate was dipped in CAMAG tank for a minute and further dried in CAMAG plate heater at 100°C ± 5°C for 3 min for development of spots. The R_f values were recorded, and photographs were taken. (6)

Result and Discussion

Organoleptic parameters

Sensory physiognomies like visual, olfactory, tactile, and taste attributes are an integral part and pilot basis for quality assurance, palatability as well as consumer's level of acceptability of the formulation. Capsule Unex has possess purple to yellow colour, ingredient specific odour, mild bitter taste of ingredients and smooth out appearance in all the three batches.

The physical properties analysis of all three batch sample is described in table 1.

Table 1: Physical properties of capsule Unex

N	Parameters	Specification	Sample (S1)	Sample (S2)	Sample (S3)	Average
1	Weight variation (%)	± 7.5 %	0.50%	0.48%	0.49%	0.49%
2	Disintegration time (min)	NMT 30 min	6 to 7 min	7 to 8 min	6 to 7 min	Avg. 6 to 8 min
3	Bulk density	0.71 to 0.81 g/ml	0.7848 g/ml	0.7986 g/ml	0.7915 g/ml	0.79 g/ml
4	Tapped Density		0.8496 g/ml	0.8512 g/ml	0.8563 g/ml	0.85 g/ml
NMT: Not more than						

The average weight variation of capsule Unex batches is within the variation of 7.5% as described by API. Less the weight variation defines appropriate amount of drugs delivery in all doses.

Physicochemical evaluation

The observations for pertaining to physicochemical measurements of capsule Unex have been represented in table 2. The pH of all the batches of capsule Unex has been found weakly acidic. A weakly acidic drug on oral administration, the major part of it remains unionized in the stomach, and diffuses across the gastric mucosa.(7) For better absorption of drugs in gastro-intestinal tract, the degree of ionization and lipid solubility serves as important parameters.

Loss on drying (LOD) is the weight loss due to resultant water and volatile matter; expressed in percentage (w/w). Moisture plays an important role in the formulation stability. If present in unallowable amount, more risk of microbial growth in the product. Moreover, moisture may also lead to the hydrolysis of hydrolysable components of the formulations. Hence, minimal or no moisture content is permissible in solid formulations. (8) The LOD of all samples are within permissible limits.

Total ash vale defines the ash left after incineration process. It comprises presence of physiological (derived from plant tissue e.g. carbonates and phosphates) and non-physiological ash (derived from external materials e.g. silicates and silica). All batch samples showed total ash value within permissible limits.

Acid-insoluble ash is insoluble in diluted hydrochloric acid; the left our material is silica or earthy materials. This value of all samples is within the limits of specification.

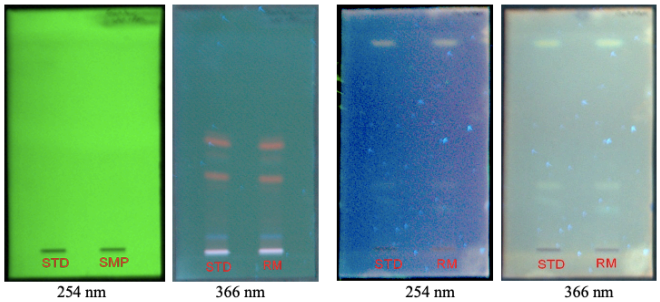
Water and alcohol-soluble extractives indicate amount of specific component soluble in aqueous and alcohol media. The value plays a pivotal role in the drug evaluation as every drug has a particular number of principal components soluble in different media. Less extractive value indicates less component concentration and suggestive of addition of exhausted material, adulterated substance or inappropriate method of formulating product. The extractive values in water and alcohol media are as per specification are described in table 2.

Table 2: Physicochemical properties of capsule Unex

N	Parameter	Specification	Sample (S1) %w/w	Sample (S2) %w/w	Sample (S3) %w/w
1	**LOD**	NMT 10 %w/w	1.42	1.86 %w/w	1.09 %w/w
2	**pH**	4 to 6	4.56	4.62	4.68
3	**Total Ash (%)**	NMT 16.3 %w/w	6.9865 %w/w	7.0215 %w/w	6.8896 %w/w
4	**Acid insoluble Ash (%)**	NMT 5.1 %w/w	1.0213 %w/w	1.1254 %w/w	1.0865 %w/w
5	**Water soluble extractives (%)**	NLT 26.0 %w/w	29.8754 %w/w	30.0215 %w/w	29.9985 %w/w
6	**Alcohol soluble extractives (%)**	NLT 11.6 %w/w	13.8254 %w/w	14.0213 %w/w	13.9945 %w/w
NMT: Not more than, NLT: Not less than					

HPTLC

Figure 1: HPTLC of *Gokshura* standard with *Gokshura* raw material sample @ 254 nm and 366 nm before and after derivitization



STD: Standard of *Gokshura*, SMP: Sample of *Gokshura* i.e. raw material, RM: Standard of raw material of *Gokshura.*

HPTLC of *Gokshura* standard sample with *Gokshura* raw material

At 254 nm, no spot was observed for both *Gokshura* standard sample (GSS) and *Gokshura* raw material (GRM). However, at 366 nm, both GSS and GRM displayed blue colour spots with absorbance values of 0.56, 0.60, and 0.76.

After derivatization, GSS and GRM showed two violet colour spots with Rf values of 0.23, 0.31, 0.46, and 0.72. (Figure 1).

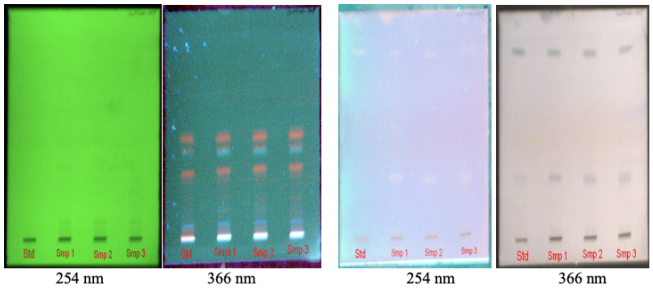
HPTLC of *Gokshura* standard sample and Unex pellet 3 batches

At 254 nm, no spots were observed for GSS and Unex pellet batch No. 1, 2 and 3. However, at 366 nm, all samples, including GSS and Unex pellet batches No.

1, No. 2, and No. 3, displayed blue colour spots with absorbance values of 0.56, 0.60, and 0.7

The absorbance results after derivatization, the spots developed violet colour at Rf 0.23,0.31,0.46,0.72 in GSS and all the three batch sample of Unex pellet. (Figure 2)

Figure 2: Comparative HPTLC of *Gokshura* standard sample with Unex pellet samples @ 254 nm and 366 nm before and after derivitization



***Gokshura* and Unex pellet 3 batches 1,2,3 Before derivatization** | ***Gokshura* and Unex pellet batches 1,2,3 After derivatization**

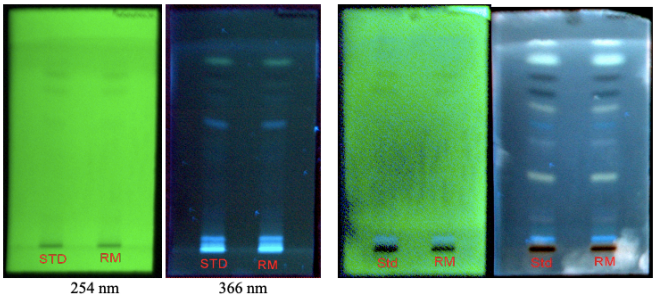
STD: Standard of *Gokshura*, SMP1: Sample 1 of Unex pellet, SMP2: Sample 2 of Unex pellet, SMP3: Sample 3 of Unex pellet.

HPTLC of *Punarnava* standard sample and *Punarnava* raw material

At 254 nm, both *Punarnava* standard sample (PSS) and *Punarnava* raw material (PRM) developed grey colour spots with absorbance values of 0.54 and 0.70. At 366 nm, both PSS and PRM exhibited blue colour spots with absorbance values of 0.56, 0.60, and 0.76.

After derivatization, for PSS and PRM developed brown colour spots with Rf values of 0.31, 0.50, and 0.75. (Figure 3).

Figure 3: Comparative HPTLC of *Punarnava* standard sample with raw material @ 254 nm and 366 nm before and after derivitization



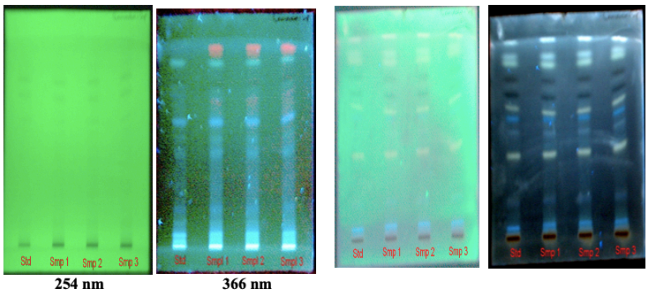
***Punarnava* and raw material Before derivatization** | ***Punarnava* and raw material After Derivatization**

STD: Standard of *Punarnava*, RM: Raw material of *Punarnava*

HPTLC of *Punarnava* standard sample with Unex pellet 3 batches

At 254 nm, all samples, including PSS and Unex pellet batches 1, 2, 3; displayed grey colour spots with absorbance values at Rf 0.54 and 0.70. Similarly, at 366 nm, all samples exhibited blue colour spots with absorbance values at Rf 0.56, 0.60, and 0.76.After derivatization absorbance showed presence of brown colour spots with absorbance values of 0.31, 0.50, and 0.75 in PSS and Unex pellet Batch No. 1, 2, 3. (Figure 4)

Figure 4: Comparative HPTLC of *Punarnava* standard sample with Unex pellet samples @ 254 nm and 366 nm before and after derivitization



***Punarnava* and Unex pellet batch 1,2, 3 Before derivatization** | ***Punarnava* and Unex pellet batch 1,2,3 After Derivatization**

STD: Standard of *Punarnava*, SMP1: Sample 1 of Unex pellet, SMP2: Sample 2 of Unex pellet, SMP3: Sample 3 of Unex pellet.

The Rf value spots observed in GSS, GRM, PSS, PRM are as described in API for the drug *Gokshura* and *Punarnava* and same observed in all the three batch samples of capsule Unex. (9) The matching spots with standard; confirms availability of active components of raw material i.e. *Gokshura* and *Punarnava.* This implies the capsule is prepared with standard materials. (Figure 1 and 3)Further, the standard raw material was traced in Capsule Unex as evident through HPTLC findings. (Figure 2 and 4)

Heavy metal analysis

Herbal medicines have been reported for having heavy metals due to contamination, soil in which it grows which aids up the accumulation of toxic heavy metals in the plant material which threats to quality of product and questions on its safety. All the three batch sample of capsule Unex was found to be free from Pb (NMT <10 ppm), Ar (NMT < 3 ppm), Cd ((NMT < 0.3 ppm) and Hg (NMT <1 ppm) which are established values for safe human consumption.

Medicinal plant materials are likely to be contaminated by pesticides due to exposure to agricultural practices of spraying, soil treatment, cultivation and administration of fumigants during storage. All batch sample of capsule Unex complies with the pesticide standards limits organochlorine (NMT 1 ppm), organo-phosphorous (NMT 1 ppm) and pyrethroids (NMT 0.3 ppm) which are below the detection limit. This signifies capsule Unex is qualitatively safe and have safe storage.

Analysis of microbial, fungal load and aflatoxin

The major threats of contamination of herbal medicine include the collection place, soil, handling, cleaning, unsuitable transportation and poor storage which likely to cause microbes infestation to drugs. Thus herbal medicines especially where underground parts are used normally likely to carry bacteria and moulds which originates in soil in high numbers. This is one of the reported shortcomings on herbal

formulations. The contaminants that present serious health hazards are pathogenic bacteria such as *Salmonella* species, *Escherichia coli*, *Salmonella*, Staphylococcus aureus, and *Shigella spp, Psedomonas aeruginosa* etc.(10) In-appropriate handling as discussed aforesaid, raises the risk of mycotoxin production, especially aflatoxin. Afaltoxin are reported to be carcinogenic, immunosuppressive, teratogenic, neurotoxic and nephrotoxic. (11), (12)

In the samples of tab Unex, total microbial count was within permissible limits (NMT 100000 cfu/g), total yeast and mould count (NMT 1000 cfu/g) and *Enterobacteriaceae* count (NMT 1000 cfu/g) also were in allowable limits. The samples were devoid of species like E. coli, *Samnonella*, *Staphylococcus*, *Psedomonas aeruginosa*. The sample compiles with the detection of aflatoxine B1 (NMT 2 ppb) and aflatoxine B1+B2+G1+G2 (NMT 5 ppb). The analysis of microbial, fungal and aflatoxin load indicates the proper collection and manufacturing and packing of capsule Unex.(Table 3)

Table 3: Analysis of microbial, fungal load and aflatoxin

N	Microorganism test	Specification	Sample (S1)	Sample (S2)	Sample (S3)
1	**Total microbial plate count**	NMT 100000 cfu/g	850 cfu/g	820 cfu/g	880 cfu/g
2	**Total yeast and mould count**	NMT 1000 cfu/g	50 cfu/g	45 cfu/g	55 cfu/g
3	***Enterobacteriaceae***	NMT 10000 cfu/g	Absent/g	Absent/g	Absent/g
4	***E. coli***	Absent/g	Absent/g	Absent/g	Absent/g
5	***Salmonella* species**	Absent/g	Absent/g	Absent/g	Absent/g
6	***Staphylococcus aureus***	Absent/g	Absent/g	Absent/g	Absent/g
7	***Psedomonas aeruginosa***	Absent/g	Absent/g	Absent/g	Absent/g
8	**Aflatoxin B1**	NMT 2 ppb	Complies	Complies	Complies
9	**Aflatoxin B1+B2+G1+G2**	NMT 5 ppb	Complies	Complies	Complies

It has been well reported that, herbs collected from various habitat and on different times of season shows variation in active phytoconstituents levels. (13), (14), (15). Hence, standardising each batch of medicinal preparation is important. The above study has targeted quality standards of raw material as well as finished product.

Tribulus terrestris L. and *Boerhavia diffusa* L. contains medicinally important phytoconstituents such as alkaloids, glycosides, flavonoids, flavonol, steroidal saponins etc. Both the herbs are testosterone booster. (16), (17), (18), (19) The wide range of pharmacological benefits of *Gokshura* and *Punarnava* are depicted in figure 1.

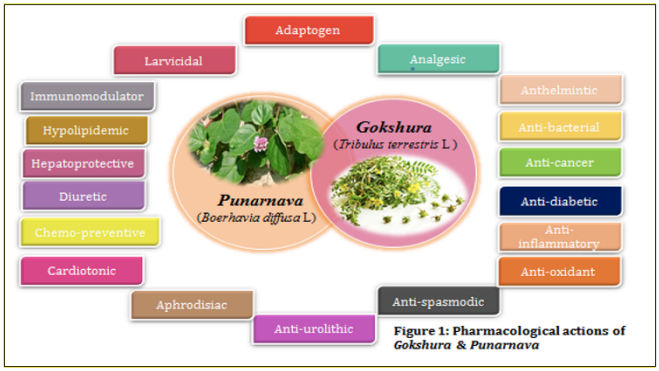


Figure 1: Pharmacological actions of *Gokshura & Punarnava*

The combination of *Gokshura* and *Punarnava* is specially designed for ureteric calculi. The clinical validation is documents. (20) Considering the broad therapeutic benefits, capsule Unex can be used for treating range of disease namely urinary infection, oedema due to renal impairment, pain, *Vatavyadhi* (diseases due to vitiation of *Vata* specifically neuro-muscular origin), liver diseases, loss of libido, male infertility symptoms etc. It can also be though for using as sports medicine and a palliative care medicine for managing side effects of chemo-radiation.

Conclusion

The study divulges that, satisfactory quality control parameters were followed for the preparation of capsule Unex. Organoleptic parameters, physical characters, physicochemical analysis, HPTCL, heavy metal analysis, pesticide residue and microbial overload analysis were carried out as per the norms of WHO guidelines. Detection of phytoconstituent's from *Tribulus terrestris* L. and *Boerhavia diffusa* L. in capsule Unex; absence of heavy metals, pesticides in raw material and microbes in the finished product indicates the genuineness of the capsule. Thus, capsule Unex represents quality parameters of Ayurvedic Pharmacopoeia of India.

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Conflicts of interest: There are no conflicts of interest.

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