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## Qualitative & quantitative phytochemical assessment of #PPL/2013 capsules – A poly-herbal formulation for gynaecological disorders

**Divya Gangolli, Rajashree Rane, Chetna Chotalia, Sachin Kundalwal, Kavita Salkar, Ashish Suthar**

### Abstract

Now-a-days, gynaecological disorders in women have become extremely common due to hormonal imbalance caused by stress, birth control pills, genetics, obesity, lack of exercise and sedentary lifestyle. This research article focuses on the qualitative & quantitative phytochemical analysis of 10 different batches of a poly-herbal capsule formulation containing Lodhra, Haldi, Yashtimadhu and Shatavari all of these being extremely useful in curing gynaecological disorders. Initially, the 10 batches were subjected to physico-chemical tests like Disintegration Time, Average weight, Uniformity of Weight, Loss On Drying, Total Ash, Acid Insoluble Ash, Water Soluble Extractive, Alcohol Soluble Extractive and pH of 1% solution (w/v) in water. Subsequently, quantitative assays like Content of Tannins and Curcumin were carried out. Also, HPTLC fingerprinting of all the batches was carried out for identification purpose. Finally, Heavy metals and Microbiological count were analysed in all the samples. The results of all the parameters of the 10 batches were within the specified limits and also showed minimum batch to batch variation. This study proves that there is consistency in the commercial production batches of PPL/2013 Capsules and the end user (patient) can rely on the quality standard of the product for safe consumption.

**Keywords:** Gynaecological Disorders, hormonal imbalance, poly-herbal, phytochemical assessment, safe consumption.

### 1. Introduction

India's traditional healthcare system is based on Indian philosophical, medicinal and psychological concepts. Its objective is to maintain or re-establish a state of well-being in an organism. The World Health Organisation has defined health as – "Health is a state of complete physical, mental and social well-being and not merely the absence of disease." The nature of health problems is changing in ways that were partially anticipated and at a rate that is wholly unexpected [1]. A female's multi-faceted life undergoes a series of physiological changes in each and every phase. Her active reproductive phase is bestowed with highest orientation of physical and psychological balance for the maintenance of harmony in her life [2]. However, very often the health issues of women (mainly gynaecological disorders) are neglected due to various reasons. The common gynaecological problems that women face are urinary tract infection, pubertal changes, post-menopausal syndrome, hot flashes, menopause, poly-cystic ovarian syndrome, bacterial vaginosis, vaginal candidiasis, infertility, menorrhagia, dysmenorrhoea & pre-menstrual mood swings, migraine or bloating. These disorders occur due to hormonal imbalances and metabolic disruptions in the female body due to various reasons like fatigue from hectic routine, emotional stress, malnutrition, life-style changes and obesity. All these factors lead to a total disarray of hormones in the body that result in disruptions in the normal menstrual cycle, infertility, hypertension, lower resistance to pathogens and ultimately gynaecological afflictions [3]. Medicinal plants have given several potent drugs to the modern pharmaceutical industry [4]. Over thousands of years, millions of women have depended on herbs, throughout their lives for their comfort, nourishment and healthcare. Throughout history, women have conveyed information from mother to daughter on how herbs can cure the different gynaecological ailments. Plants are sometimes referred to as being phyto-oestrogenic or phyto-progesteronic. This is because some plants have molecular structures similar to the hormones oestrogen and progesterone. They can occupy the receptor sites in the body that would normally be taken up by these hormones. Some plants can both increase or decrease hormonal levels in the human system [5]. Many synthetic drugs are

Known to act on a single molecular target and provide symptomatic relief. The multi-target responses of herbal drugs prove to be beneficial in chronic conditions, gynaecological disorders and in restoring health status. #PPL/2013 is marketed as Rtnorm Capsules in India and Normocycle Capsules in Ukraine. This research article focuses on the detailed study of a poly-herbal formulation – PPL/2013 Capsule that is a unique formulation of powerful herbs that act together as an effective treatment for the common gynaecological ailments observed in women. The herbal ingredients present in PPL/2013 capsule balance female hormones and regulate menstrual cycle naturally without any adverse effects. PPL/2013 capsule is useful in relieving all types of discomforts experienced by women before and during menstruation.

It corrects excessive vaginal discharge, cures profuse bleeding during menstruation and relieves discomforts that are experienced by women during monthly menstrual cycle. The ingredients of the capsule include extracts of *Symplocos racemosa* (Lodhra), *Glycyrrhiza glabra* (Yashtimadhu), *Asparagus racemosus* (Shatavari) and *Curcuma longa* (Haldi). *Symplocos racemosa* (Lodhra) is an evergreen Ayurvedic plant from the family Symplocaceae. It is a widely used herbal remedy mainly for gynaecological disorders. It is used in the Indian system of medicine as a single drug or in multi-component preparations. Its bark is used for menorrhagia and other uterine disorders [6]. It is useful in inflammation of uterus and reduces leucorrhoea & menorrhagia (relaxes the uterine tissue) [7]. Its decoction is used in diseases of uterus [8]. Mamata Jadhav *et al* [9] have reported that *S.racemosa* decreases testosterone & cholesterol levels and increases estrogen & progesterone levels that helps in the treatment of Poly-cystic Ovarian Syndrome. The plant helps in maintaining the ratio of estrogen: progesterone in the female body thus preventing menstrual irregularity. The phytoestrogens in Lodhra ameliorate mild to moderate pre and post menstrual depression by acting on the serotonergic systems. Saraswathi *et al.* [10] evaluated the ethanolic extract of Lodhra bark in treating female reproductive dysfunctions. *Glycyrrhiza glabra* (Yashtimadhu) is a herbaceous perennial plant of the family Fabaceae. The health components of this herb possess anti-spasmodic, anti-inflammatory and a mild estrogenic activity which helps to treat menstrual cramps. This also helps to get relief from symptoms of PMS like mood swings, breast tenderness, nausea and bloating. It is recognized as a herb that balances the menstrual cycle naturally [11]. *Asparagus racemosus* (Shatavari) belonging to family Liliaceae is a climbing plant which grows in low jungle areas throughout India [12]. “Shatavari” literally means who possesses a hundred husbands or acceptable to many. Ayurveda has called Shatavari as the “Queen of herbs” and is the primary herb recommended for female health [13]. It is a foetal tonic, galactogogue and aphrodisiac. It is a main rejuvenative tonic for females. It works as a rasayana in threatened abortion, menorrhagia and suppressed lactation [14]. Shatavari roots are useful in leucorrhoea and agalactia [15]. Steroidal compounds known as saponins exert hormone like actions and are extremely beneficial in the treatment of many known gynecological problems related to hormonal imbalances in the body of the women. Shatavari is mainly known for its phyto-estrogenic properties and has developed much interest after the increasing awareness about the harmful effects of synthetic estrogens. It is extremely beneficial in correcting menstrual irregularities in women like dysmenorrhoea, pre-menstrual

syndrome, irregular bleeding and also balances the pH of the cervical area [16]. *Curcuma longa* (Haldi) is a rhizomatous herbaceous tropical perennial plant from the ginger family Zingiberaceae. It is a gold colored spice commonly used in the Indian subcontinent. Haldi is used extensively as a household remedy for various diseases including menstrual disorders. It is administered in post-partum period due to its purifying effect on uterus & breast milk [17]. Rhizomes are useful in amenorrhoea [18]. The active ingredient is 3% dicinnamoylmthene derivatives (95% curcuminoides). Studies show that it increases the menstrual flow. It seems to have a cyclo-oxygenase (COX) like effect which is responsible for treating dysmenorrhoea. It acts as an anti-spasmodic and thus reduces menstrual cramping. It helps to regulate the activities of the female reproductive system, helps in balancing the hormone levels & purifying the uterus by removing the waste blood in the form of menstruation, thus treating irregular periods [19]. There is a need to assure the safety of herbal formulations in order to acquire their maximum benefits even though these have been proven to be efficacious in pharmacological studies or by clinical evaluation. Although many plant extracts used traditionally have passed the test of time in terms of toxicity and adverse effects, the safety of the active phytochemicals present in plants must precede their pharmaceutical use [20]. Hence, detailed phytochemical assessment of 10 different batches of PPL/2013 Capsules was carried out. This included physico-chemical parameters like Description, Disintegration Time, Average weight of capsules, Average Filled weight of capsules, Uniformity of Weight, Loss on Drying at 105 °C, Total Ash, Acid Insoluble Ash, Water Soluble Extractive, Alcohol Soluble Extractive and pH of 1% w/v solution in water. Quantitative Assays like Content of Tannins and Curcumin were carried out as they are the major functional groups responsible for the relief from gynaecological disorders. The consistency in these test results provides an assurance to the physician, pharmacist and the consumer that the product will perform uniformly and depict homogeneity in a manner that is satisfactory for the purpose for which it is recommended, thus making it reliable to be used as medicine. This helps in the detection of inferior or deviating batches if any. HPTLC fingerprinting was carried out to qualitatively prove the presence of each individual ingredient that is mentioned on the label, in the capsule formulation. Impurities in pharmaceutical drugs are of great concern not only due to the inherent toxicity of certain contaminants, but also due to their adverse effect on drug stability and shelf life. This necessitates the monitoring of organic and inorganic impurities in the drugs before consumption. The detection of trace elemental impurities in the drugs is of great importance thus, Heavy Metal testing in all the batches was carried out. Also, microbiological testing of all the samples was carried out to check the safety of the capsules for patient administration.

## 2. Materials and Methods

### 2.1 Raw materials

Raw materials used in the manufacture of all the batches of PPL/2013 Capsules were procured from authorized vendors in India. The ten PPL/2013 capsule batches were prepared in the commercial manufacturing plant of Piramal Phytocare Ltd.

### 2.2 Reagents

All the chemicals and reagents used for manufacturing as well as testing purposes were of AR grade and were purchased from M/s Qualigens and M/s Merck India Ltd.

### 2.3 Glassware and Instruments

All the glassware used was well calibrated and procured from M/s Borosil Glassworks Ltd. Instruments used were Disintegration Time Apparatus (M/s Thermonik), Electric oven (M/s Pathak Electrical Works Ltd.), weighing balance (M/s Shimadzu Corporation), hot plate (M/s Scientific Sales Syndicate), pH meter (M/s Control Dynamics), UV Spectrophotometer (M/s Shimadzu) & HPTLC (M/s Anchrome, M/s Camag).

### 2.4 Physico-chemical Methods

The parameters Description, Disintegration Time, Average Weight of Capsules, Average Net Filled Weight of Capsules & Uniformity of Weight were carried out as per Indian Pharmacopoeia [21]. Loss on Drying at 105 °C, Total Ash, Acid Insoluble Ash, Water & Alcohol Soluble Extractives and pH of 1% solution in water were carried out as per Ayurvedic Pharmacopoeia of India [22].

### 2.5 Qualitative & Quantitative Assays

The Content of Tannins [23] and Content of Curcumin [24] was analysed by the Spectrophotometric method.

HPTLC technique was applied to qualitatively determine the

presence of all the ingredients in each batch of the capsules. The conditions applied for HPTLC were as follows: Concentration of sample → Blend of 1 capsule/10 ml (methanol), Concentration of Reference Standards → 25 mg/10 ml, 20 mg/10 ml, 10 mg/10 ml & 5 mg (methanol) extracts of Lodhra, Shatavari, Yashtimadhu & Haldi respectively, Stationary Phase → TLC Aluminium sheets silica gel 60 F 254, Solvent system used → Chloroform : Methanol (9:1), Sample Volume → 10 microlitre, Saturation Time → 30 minutes, Spotting level → 1 cm, Time of Run → 20 minutes, Length of Run → 8.0 cm, Detection at → 254 nm and 366 nm and visual detection by spraying Vanillin – Sulphuric acid Reagent (VSR).

### 2.6 Heavy Metal testing

Heavy Metal testing was carried out on Inductively Coupled Plasma (ICP) spectrometry, to determine the values as per AYUSH Guidelines.

### 2.7 Microbiological Testing

Microbiological testing was performed as per USP/BP guidelines.

## 3. Results

**Table 1:** Results of Physico-chemical parameters, Assays & HPTLC fingerprinting of Batches 1 to 5

Test Parameters	Inhouse Specifications	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Description	“0” size capsule filled with yellowish-brown colored powder	Complies	Complies	Complies	Complies	Complies
Disintegration Time (minutes)	NMT 30 minutes	16	12	14	14	15
Average weight of Capsules (mg)	About 500 mg $\pm$ 7.50%	494.87	495.83	496.28	495.54	508.77
Average filled weight of Capsules (mg)	About 405 mg $\pm$ 7.50%	401.76	401.74	403.70	401.45	416.40
Uniformity of Weight	+7.5% of Avg. Filled weight of capsule	Complies	Complies	Complies	Complies	Complies
Loss On Drying at 105°C (% w/w)	NMT 15.00% (w/w)	6.75	5.68	6.00	5.39	6.43
Total Ash (% w/w)	NMT 20.00% (w/w)	7.98	7.81	7.89	8.07	8.07
Acid Insoluble Ash (% w/w)	NMT 10.00% (w/w)	5.47	5.45	6.26	5.07	5.35
Water Soluble Extractive (% w/w)	NLT 30.00% (w/w)	60.09	64.66	62.50	65.05	62.70
Alcohol Soluble Extractive (% w/w)	NLT 4.00% (w/w)	7.94	6.63	7.04	7.01	7.41
pH of 1% solution in water	4.00 to 7.00	4.65	4.85	4.85	4.86	5.18
Content of Tannins (mg/capsule)	NLT 4.50 mg/capsule	11.36	12.15	12.21	12.14	12.03
Content of Curcumin (mg/capsule)	NLT 4.00 mg/capsule	5.90	5.91	5.79	5.95	5.97
HPTLC fingerprinting	To pass the test – Complies/Does Not Comply	Complies	Complies	Complies	Complies	Complies

NMT: Not More Than

mg: Milligram

% w/w: percentage weight by weight

NLT: Not Less Than

**Table 2:** Results of Physico-chemical parameters, Assays & HPTLC fingerprinting of Batches 6 to 10

Test Parameters	Inhouse Specifications	Batch 6	Batch 7	Batch 8	Batch 9	Batch 10
Description	“0” size capsule filled with yellowish-brown colored powder	Complies	Complies	Complies	Complies	Complies
Disintegration Time (minutes)	NMT 30 minutes	16	15	16	20	14
Average weight of Capsules (mg)	About 500mg $\pm$ 7.50%	507.46	504.28	510.38	503.76	499.15
Average filled weight of Capsules (mg)	About 405mg $\pm$ 7.50%	414.70	411.80	417.87	409.03	405.64
Uniformity of Weight	+7.5% of Avg. Filled weight of capsule	Complies	Complies	Complies	Complies	Complies
Loss On Drying at 105°C (% w/w)	NMT 15.00% (w/w)	6.60	6.11	6.00	6.17	5.35
Total Ash (% w/w)	NMT 20.00% (w/w)	8.13	8.26	8.19	8.24	8.39
Acid Insoluble Ash (% w/w)	NMT 10.00% (w/w)	5.36	5.56	5.20	5.06	5.22
Water Soluble Extractive (% w/w)	NLT 30.00% (w/w)	61.59	63.08	61.41	56.68	58.66
Alcohol Soluble Extractive (% w/w)	NLT 4.00% (w/w)	7.49	7.10	7.19	6.94	6.60
pH of 1% solution in water	4.00 to 7.00	5.16	5.14	5.09	5.18	5.16
Content of Tannins (mg/capsule)	NLT 4.50mg/capsule	11.86	11.78	11.83	10.89	10.80
Content of Curcumin (mg/capsule)	NLT 4.00mg/capsule	5.31	5.34	5.25	5.20	5.16
HPTLC fingerprinting	To pass the test – Complies/Does Not Comply	Complies	Complies	Complies	Complies	Complies

NMT: Not More Than

mg: Milligram

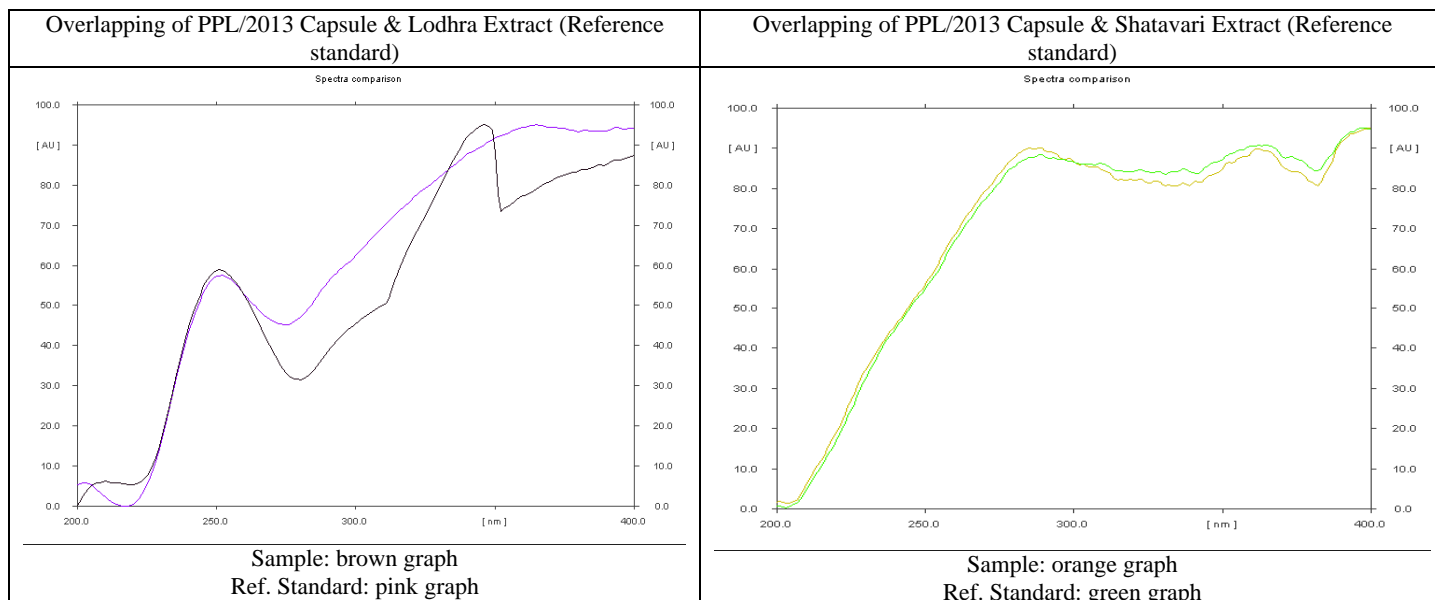
% w/w: percentage weight by weight

NLT: Not Less Than

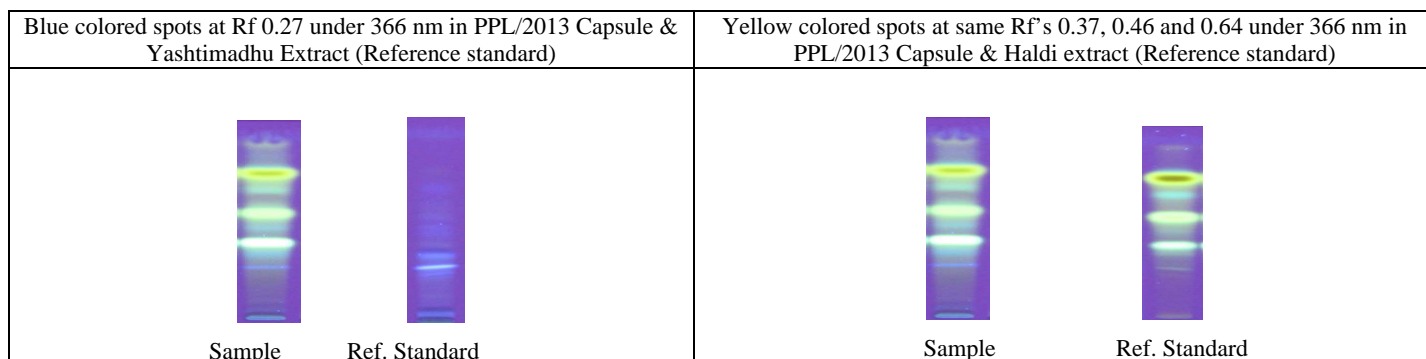
**Table 3:** Results of Heavy Metal Testing & Microbiological Testing of Batches 1 to 10

Test Parameters	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9	Batch 10
<b>Heavy Metals → As Per Department of AYUSH</b>										
<b>Arsenic (As) in ppm</b>	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2
<b>Lead (Pb) in ppm</b>	Less than 0.2	0.201	Less than 0.2	0.739	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	0.256	0.309
<b>Mercury (Hg) in ppm</b>	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2
<b>Cadmium (Cd) in ppm</b>	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2	Less than 0.2
<b>Microbiological Testing → As per USP/BP Guidelines</b>										
<b>Total aerobic microbial count (cfu/g)</b>	10	40	40	30	120	390	60	60	10	80
<b>Total combined yeast/ moulds count (cfu/g)</b>	<10	<10	<10	<10	40	30	<10	<10	<10	20
<b>Bile-tolerant Gram negative bacteria (cfu/g)</b>	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
<b>E.coli</b>	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
<b>Salmonella species</b>	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
<b>S.aureus</b>	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
<b>P.aeruginosa</b>	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
<b>Clostridium species</b>	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

ppm: parts per million      cfu/g: colony forming units per gram



**Fig 1:** HPTLC fingerprinting profile of Lodhra, Shatavari and PPL/2013 Capsules by overlapping at 254nm



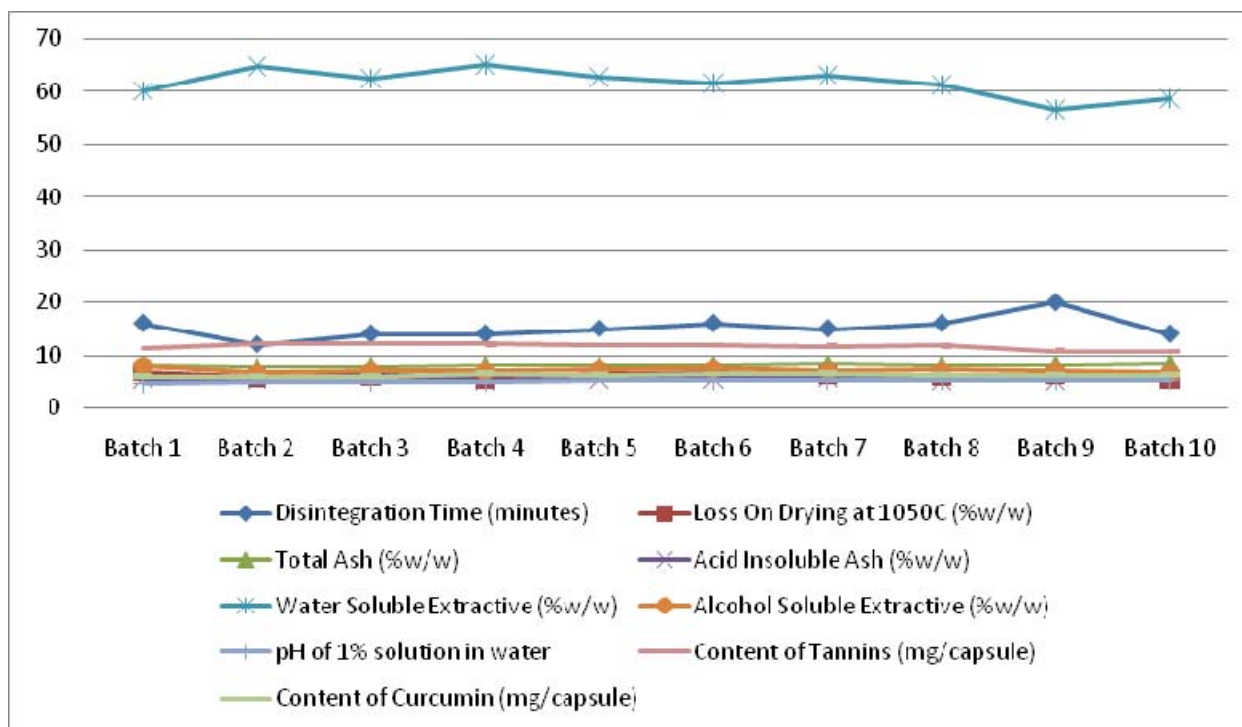
**Fig 2:** HPTLC fingerprinting profile of Yashtimadhu, Haldi and PPL/2013 Capsules by observing the plate under 366nm

**4. Conclusion**

The results of Description, Disintegration Time, Average Weight of Capsules, Average Filled Weight of Capsules, Uniformity of Weight, Loss On Drying at 105 °C, Total Ash, Acid Insoluble Ash, Water Soluble Extractive, Alcohol Soluble Extractive and pH of 1% w/v solution in water are

well within the specified Inhouse specifications.

The graph given below depicts the consistency (minimum deviation) in the results of all the physico-chemical parameters and quantitative assays that shows the precision in the manufacturing procedure of all the batches.



The practical values of Content of Curcumin in the 10 samples are 118.00%, 118.20%, 115.80%, 119.00%, 119.40%, 106.20%, 106.80%, 105.00%, 104.00% and 103.20% of the theoretical value, respectively. The practical values of Content of Tannins with respect to theoretical values are also high, but they are much higher than the expected values (more than 120%) possibly due to other sources of tannins in the constituents of the sample that cannot be standardised.

The practical values of assay contents being in the range of 80-120% (higher in case of Tannins) proves the accuracy in the quantitative assay results of all the 10 batches which in turn depicts regularity in the product quality and correctness in the manufacturing process.

Figure 1 shows the overlapping spectrum of the PPL/2013 capsule with the reference standards of *Symplocos racemosa* (Lodhra) and *Asparagus racemosus* (Shatavari). Figure 2 shows the matching blue and yellow colored spots of PPL/2013 capsule and the reference standards of *Glycyrrhiza glabra* (Yashtimadhu) and *Curcuma longa* (Haldi) respectively after observing the plate under 366nm. This proves the presence of all the four constituents in all the 10 batches of the capsules.

Heavy Metal results comply as per AYUSH guidelines (Arsenic: Not More Than 3.0 ppm, Lead: Not More Than 10ppm, Mercury: Not More Than 1ppm and Cadmium: Not More Than 0.3 ppm). This proves that all the samples are free from trace elemental impurities.

Also, the results of Microbiological analysis are within USP/BP guidelines [Total aerobic microbial count: Not More Than 10000cfu/g, Total combined yeast/moulds count: Not More Than 1000cfu/g, Bile Tolerant Gram negative bacteria: Not More Than 100cfu/g, and Pathogens (E. coli and Salmonella species should be absent in 10 g sample) and (*S. aureus*, *P. Aeruginosa* and *Clostridium* species should be completely absent in the sample.)] This shows that the samples are free from microbiological load and thus are safe for consumption.

Quality Management ensures superior quality and reliability of products. Quality is an extremely crucial parameter that differentiates a drug manufacturer from its competitors. The

standards of a drug should not only meet, but exceed customer (patient) satisfaction, which leads to customer loyalty and eventually increased revenue and higher productivity. Quality Control and management ensures continuous improvement and elimination of defects in production of drugs. In this study a very important aspect of Quality Control of drugs that is the complete Qualitative and Quantitative Phytochemical Evaluation of commercial batches of Finished Product (PPL/2013 Capsules) is carried out. The accuracy and precision in the results of 10 batches of the product confirms that the quality of the product has been maintained and assures the patient its safe and secure administration.

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