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Standardization of marketed cystone tablet: A herbal formulation

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Abstract

The Present study aims to standardize Cystone tablet based upon chromatographic and spectral studies. The spectral data and HPTLC fingerprint of ethanolic extract of Cystone formulation could be used as a valuable analytical tool in the routine standardization of tablet to check the batch to batch variation. The ethanolic extract of Cystone tablet were compared by TLC, HPTLC and HPLC analysis to evaluate the presence of catechin employing Toluene: Ethyl acetate: acetone (2:4:4 v/v/v), as a mobile phase respectively. The R_f values (0.80) for catechin in both sample and reference standard were found comparable under UV light at 366 nm respectively. The high performance thin layer chromatography method developed for quantization was simple, accurate and specific. The present standardization provides specific and accurate tool to develop qualifications for identity, transparency and reproducibility of biomarkers catechin tablet formulation.

Keywords: Standardization, cystone tablet, Markers

1. Introduction

Ayurveda is considered by many scientists to be the oldest healing science. In Sanskrit, Ayurveda means "The Science of Life." Ayurvedic knowledge originated in India more than 5,000 years ago and is often called the "Mother of All Healing" [1]. Ayurveda translates into knowledge (Veda) of life (Ayur) and is one of the oldest and still widely practiced medical systems in the Indian subcontinent [2]. The concept of Ayurvedic medicine is to promote health, rather than to fight disease, and Ayurveda in daily life aims at maintaining harmony between nature and the "individual" to ensure optimal health [1]. Ayurveda contains 8 branches of sciences and 10 different diagnostic tools based on tridosha theory (three humours of body). Ayurveda comprises of various types of medicines including the fermented forms namely arishtas (fermented decoctions) and asavas (fermented infusions). These are regarded as valuable therapeutics due to their efficacy and desirable features.

Bergenia ligulata belongs to family saxifragaceae. Pashanbheda, Pashana, Zakhmehayat, Asmaribheda, Ashmabhid, Ashmabhed, Nagabhid, Upalbheda, Parwatbhed and Shilabhed are the common name of *Bergenia ligulata*. It is called Stone breaker because it dissolves slabs. Rhizome is the medicinally used part of this. The plant *Bergenia ligulata* is main botanical source of Pashanbheda drug which is used in indigenous system of medicine [3-7].

2. Material and methods

2.1 Chemicals

Chloroform, formic acid, ethyl acetate, toluene were purchased from Merck, India. Methanol and ethanol of analytical reagent grade (Merck, Darmstadt, Germany) were used. catechin reference standard were purchased from Sigma-Aldrich GmbH, Germany. All other solvents and chemicals were of the highest analytical grade.

2.2 Apparatus

All the solvents purchased from E. Merck and S.D. Fine Chemicals, Mumbai. All solvents used for extraction, TLC and HPTLC studies were distilled before use. Solvents used for UV and IR studies were of spectroscopy grade. Solvents used for HPLC analysis were of HPLC grade. Precoated silica gel GF-254 plates procured from E. Merck, Mumbai were used for TLC and HPTLC studies. The UV spectra were recorded on a JASCO V 530 spectrophotometer. The FT IR spectra were recorded on JASCO FT IR 410. Atronic HPTLC system consisting of Sparylin spotting, elite-miniluminance photo documentation and CAMAG scanner. The HPLC analyses were done on a TOSOH-CCPM system. All the results are obtained by repetition of the each experiment at least three times.

2.3 Procurement of drug

Commercially available brand (M/S Himalaya Pharma) of Cystone was procured from local market.

2.4 Standardization using physicochemical parameters

The sample of Cystone was analyzed for various parameters such as Organoleptic evaluation, Physical evaluation, Moisture content etc.

2.5 Physiochemical parameters

Organoleptic properties

Appearance, color, smell, and taste were evaluated.

2.6 Friability test

Friability test apparatus Roche's friabilator (Labinda mod. no. 1020) was used for determination of friability of tablet. This device subjected the tablet to the combined effect of abrasion and hock in a public chamber and dropping the tablets at a height of 6 inches in each revolution. Weighed tablets were placed in friabilator revolving at 25 rpm for 100 revolutions. Tablet was de-dusted using a soft muslin cloth and weighed.

$$F = (W_1 - W_2 / W_1) \times 100$$

Where; (W_1 = Initial weight of tablets, W_2 = Final weight of tablets)

2.7 Tablet hardness test

Randomly three tablets were pickup and they were individually tested for the hardness by Monsanto hardness tester (Shital scientific industries Sr. no. 11012010) in terms of kg/cm.

2.8 Disintegration test

Disintegration testing apparatus (Thermonik: Mod. no. TD 20S) was used for determination of disintegration time.

2.9 Uniformity of diameter

Diameter of three randomly selected tablets was measured individually using a Vernier Caliper (UTTAR, IME type 6 inch/15 cm) and expressed in mm.

3 Extractive value (Soxhlet apparatus)

3.1 Successive extractive value

The coarse powder of *QT* was extracted successively using soxhlet apparatus with different solvent, in increasing order of polarity, petroleum ether → benzene → chloroform → ethanol. 10 g powdered drug was taken and subjected to successive extraction with each solvent for 6 h. The extracts were filtered using filter paper (Whatman No. 1) and dried on water bath. The extractive values were determined with reference to the weight of the drug taken (w/w). The procedure was repeated 3 times to calculate mean extractive values.

3.2 Non successive extractive value

The coarse powder of *QT* was extracted separately in different solvent (water, ethyl alcohol and petroleum ether) using soxhlet apparatus. 10 g powdered drug was taken and subjected to separate extraction with each solvent. The extracts were filtered using filter paper (Whatman No. 1) and evaporate on water bath. Extractive values were determined with reference to drug taken (w/w).

3.3 Ash value

Total ash and water soluble ash were done by method

mentioned in protocol for testing. Acid insoluble ash and sulphated ash were done by method mention in AP;

3.4 Loss of weight on drying at 105 °C

Loss of weight on drying (LOD) at 105 °C was done by method mention in AP.

3.5 pH value

pH value of 1% solution and pH value of 10% solution was determined as per the method mentioned in physiochemical standardization of Unani Medicine part IV.

3.6 Weight variation

Twenty tablets were selected randomly from selected batch and weighed individually. Average weight was calculated, and individual weights were compared to average weight. If not more than 2 tablets are outside the percentage limit, tablets meet the USP test (USP weight variation test).

3.7 Preparation of extracts

100 gm of the Cystone tablet powdered material was extracted with 250ml methanol and ethanol in a soxhlet extractor at a temperature of 45-50 °C for 48 hours. The extract obtained was then concentrated under reduced pressure using rotary evaporator which concentrates bulky solution down to small volumes, without bumping, at temperatures between 30 and 40 °C.

3.8 Qualitative chemical examination

The ethanolic and methanolic extracts were qualitatively evaluated by chemical tests and TLC studies for the presence of various phytoconstituents like alkaloids, carbohydrates, saponins, phenolic compounds and tannins, phytosterols and anthraquinone glycosides [8].

3.9 Isolation of Catechin by pre-para tine TLC

This extract was dissolved in methanol and resulting solution was used for preparative thin layer chromatography. For that purpose TLC plates of size 15.2×20.2 were used. Better resolution of catechin was obtained in the solvent system of Toluene: Ethyl acetate: acetone (2:4:4 v/v/v).

The developed preparative TLC was showed in [Figure no. 2]. The Rf value of catechin was calculated. After proper resolution, the spot of catechin was observed in UV chamber. The developed preparative TLC in UV chamber was showed in [Figure no. 3]. Using sharp pointer the spot was isolated. The isolated catechin was subjected for phytochemical test. The retention factor is defined as the distance travelled by the solute divided by distance travelled by the solvent.

4. HPTLC studies

HPTLC fingerprint of ethanolic extract was recorded at 366 nm. Ethanolic extract were subjected to HPTLC studies to develop fingerprints using same conditions as used for TLC.

5. Spectral studies

UV, IR and fluorescence spectra were recorded for extract. UV spectra were recorded in ethanol. IR spectra were recorded of neat sample.

6. HPLC studies

Isolated compound indicated presence of Catechin which is reported to be a major active component of Cystone formulation. Extract was analyzed by HPLC using following conditions:

Column: C18 (25 cm×4.6 mm, i.d.), 10 μ m
 Mobile phase: methanol: water (60:40)
 Detection: at 254 nm
 Flow rate: 1 ml/min

7. Results and discussion

Standardization of Cystone as per pharmacopoeia was carried out based on the physicochemical parameters [16]. The marketed sample of Cystone was found to pass all the pharmacopoeial tests (Table 1).

7.1 Organoleptic evaluation

Appearance: Circular uncoated tablet (slightly biconvex); Colour: Yellow; Smell: Rosy; Taste: Aromatic and bitter; Texture: Hard.

The mean value of friability (%), hardness (kg/cm), disintegration time (minutes) and diameter (mm) of QT. Were determined and the values are depicted in [Table 1].

Table 1: Organoleptic evaluation of Cystone tablet.

Parameter	Mean
Friability (%)	0.08 \pm 0.0053
Hardness (kg/cm)	8.1 \pm 0.082
Disintegration time (minutes)	25.80 \pm 0.453
Diameter	12 \pm 0.1

Table 2: Water soluble extractive value of Cystone tablet. *n=3

Sample	Wt. of sample (g)	Mean water soluble extractive value* (g)	Water soluble extractive value (% w/w)	\pm SD
Cystone tablet	5	0.64	12.8	0.0143

7.2.3 Determination of alcohol soluble extractive value

For the determination of alcohol soluble extractive value, 5g of Cystone tablet powdered material was weighed and taken into the conical flask in which 100ml of ethanol was added separately and allowed to macerate for 24 hours, shaking frequently for first 6 hours, then allowing to stand for next 18

7.2 Physical evaluation: [9-12]

7.2.1 Determination of solvent extractive values

Solvent extractive value is the amount of active constituent in a specified weight of medicinal plant material when extracted with specific solvent. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used.

The solvent extractive value can be determined by following methods to measure the water soluble extractive, alcohol soluble extractive and hexane soluble extractive value using water, ethanol and hexane as solvent for extraction respectively.

7.2.2 Determination of water soluble extractive value

For the determination of water soluble extractive value, 5 g of Cystone tablet powdered material was weighed and taken into the conical flask in which 100ml of water was added separately and allowed to macerate for 24 hours, shaking frequently for first 6 hours then allowing to stand for next 18 hours, which was then filtered rapidly and 25ml of the filtrate was allowed to evaporate to dryness in a tared 250ml beaker at 105 $^{\circ}$ C. The difference in weight of the beaker is an indication of water soluble extractive value of that drug with respect to the amount of drug (5 g) taken for extraction. The obtained results are tabulated in [Table no. 2].

hours, which was then filtered rapidly and 25ml of the filtrate was allowed to evaporate to dryness in a tared 250ml beaker at 105 $^{\circ}$ C. The difference in weight of the beaker is an indication of alcohol soluble extractive value of that drug with respect to the amount of drug (5g) taken for extraction. The obtained results are tabulated in [Table no. 3].

Table 3: Alcohol soluble extractive value of Cystone tablet. *n=3

Sample	Wt. of sample (g)	Mean alcohol soluble extractive value* (g)	Alcohol soluble extractive value (% w/w)	\pm SD
Cystone tablet	5	0.48	9.6	0.01155

7.2.4 Determination of hexane soluble extractive value

For the determination of hexane soluble extractive value, 5g of Cystone tablet powdered material was weighed and taken into the conical flask in which 100ml of hexane was added separately and allowed to macerate for 24 hours, shaking frequently for first 6 hours then allowing to stand for next 18 hours, which was then filtered rapidly and 25ml of the filtrate

was allowed to evaporate to dryness in a tared 250ml beaker at 105 $^{\circ}$ C. The difference in weight of the beaker is an indication of hexane soluble extractive value of that drug with respect to the amount of drug (5 g) taken for extraction. The obtained results are tabulated in [Table no. 4].

In the determination of all extractive values, the percentages were determined with respect to the air dried material.

Table 4: Hexane soluble extractive value of Cystone tablet. *n=3

Sample	Wt. of sample (g)	Mean hexane soluble extractive value* (g)	Hexane soluble extractive value (% w/w)	\pm SD
Cystone tablet	5	0.55	11	0.00577

7.2.5 Determination of ash values

The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drug results in an ash residue consisting of an inorganic material (metallic salts and silica) in certain drugs, the percent

variation of the weight of ash from sample to sample is very small and any marked difference indicates a change in quality. The ash value can be determined by three different methods to measure the total ash, the acid insoluble ash and the water soluble ash.

7.2.6 Determination of total ash content

The total ash was determined by placing 2 g of Cystone tablet accurately powdered material into the tared crucible. Then Cystone tablet sample was placed in muffle furnace and the sample was ignited by gradually increasing the heat up to 500-600°C until it is white, indicating the absence of carbon. The crucible was kept in desiccator and weighed. Initially

carbon free ash was not obtained thus the cooled crucible was removed and the residue was moistened with about 2ml of water, dried on water bath, and ignited to constant weight. The residue was allowed to cool in desiccator for 30 minutes. Content of total ash was calculated with reference to the amount of Cystone tablet taken. The obtained results are tabulated in [Table no. 5].

Table 5: Determination of total ash content of Cystone tablet. *n=3

Sample	Wt. of sample (g)	Mean total ash content*(g)	Total ash content (% w/w)	± SD
Cystone tablet	2	0.25	12.5	0.010

7.2.7 Determination of acid insoluble ash value

The total ash which was obtained in the previous step was boiled with 25ml of 2 M HCl for 5 minutes, then it was filtered through ash-less filter paper, the insoluble matter was collected in a previously tared crucible, then the residue was

washed with hot water, then ignited in muffle furnace at about 450 °C for 30 minutes, cooled in desiccator and weighed. The percent of acid insoluble ash was calculated with reference to the amount of Cystone tablet taken. The obtained results are tabulated in [Table no. 6].

Table 6: Determination of acid insoluble ash value of Cystone tablet. *n=3

Sample	Wt. of sample (g)	Mean acid insoluble ash value* (g)	Acid insoluble ash value (% w/w)	± SD
Ash	1	0.05	5	0.011

7.2.8 Determination of water soluble ash

In the crucible containing total ash, 25ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ash less filter paper. This was then washed with hot water and ignited in a crucible for 15 minutes at a temperature

not exceeding 450°C separately. Then the weight of this residue was subtracted from the weight of total ash. Finally the content of water soluble ash with reference to the amount of Cystone tablet taken was calculated. The obtained results are tabulated in [Table no. 7].

Table 7: Determination of water soluble ash value of Cystone tablet. *n=3

Sample	Wt. of sample (g)	Mean water soluble ash value* (g)	Water soluble ash value (% w/w)	± SD
Ash	1	0.04	4	0.0101

7.2.9 Determination of moisture content

Method of determination of moisture content includes the loss on drying. It can be carried out either by heating at 100^o-105^oC or in a desiccator over phosphorus pentoxide under

atmospheric pressure at room temperature for specific period of time.

The moisture content of Cystone tablet was found to be 6.66 w/w. The obtained results are tabulated in [Table no. 8].

Table 8: Determination of moisture content of Cystone tablet. *n=3

Sample	Wt. of sample (g)	Mean moisture content * (g)	Moisture content (% w/w)	± SD
Cystone tablet	1.5	0.10	6.66	0.0115

7.2.10 Loss on drying

Loss on drying is the loss of mass expressed as percent w/w. To estimate the loss on drying 2-5 g of air dried drug is accurately weighed in dried and tared flat weighing bottle. The substance is dried to a constant mass or for the prescribed time as specified.

1.5g of sample of Cystone tablet was accurately weighed in separate dried and tared petridish. Sample Cystone tablet was placed in an oven, and drying of sample was carried out at 105°C until a constant mass of the sample was not observed. The difference in weight before and after heating in oven was

recorded and loss on drying was expressed as percent w/w.

7.2.11 Determination of moisture content by Analytical Moisture Balance

Method of determination of moisture content includes the loss on drying. It can be carried out by using Analytical Moisture Balance (Sartorius Moisture Balance Model -MA 150). The difference in weight before and after heating was recorded and loss on drying was expressed as percent w/w. The obtained results are tabulated in [Table no. 9].

Table 9: Determination of moisture content of Cystone tablet by Analytical Moisture Balance. *n=3

Sample	Wt. of sample (g)	Mean moisture content * (g)	Moisture content (%LR)	Loss of Residue (%R)
Cystone tablet	2	0.051	2.60	97.47

Ethanollic and Methanolic extract of Cystone formulation were subjected to qualitative chemical investigation. A summary of the qualitative analysis is given in Table 10.

Table 10: Qualitative chemical evaluation of extracts

Serial number	Phytoconstituents	Extract	
		Ethanol	Methanol
1	Alkaloids	+	+
2	Steroids	-	-
3	Flavonoids	+	+
4	Saponins	-	-
5	Tannins	+	+
6	Cardiac glycoside	-	-
7	Anthraquinone glycoside	-	-
8	Coumarins glycoside	-	-

(+) Indicates presence, (-) indicates absence. The chemical evaluation reported here is for the entire extract and not for the individual spot.

After isolation process the crude dried extract was obtained. This ethanolic extract was dissolved in methanol and resulting solution was used for preparative thin layer chromatography. For that purpose TLC plates of size 15.2×20.2 were used. Better resolution of catechin was obtained in the solvent system of Toluene: Ethyl acetate: acetone (2:4:4 v/v/v). The developed preparative TLC was showed in [Figure no. 1]. The Rf value of catechin was calculated. After proper resolution, the spot of catechin was observed in UV chamber. The developed preparative TLC in UV chamber was showed in [Figure no. 2]. Using sharp pointer the spot was isolated. The isolated catechin was subjected for phytochemical test. The retention factor is defined as the distance travelled by the solute divided by distance travelled by the solvent.

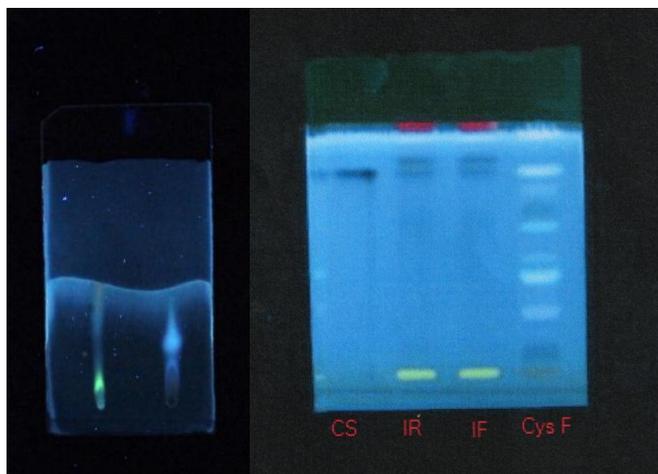


Fig 1: TLC study of Isolated and Standard Catechin.

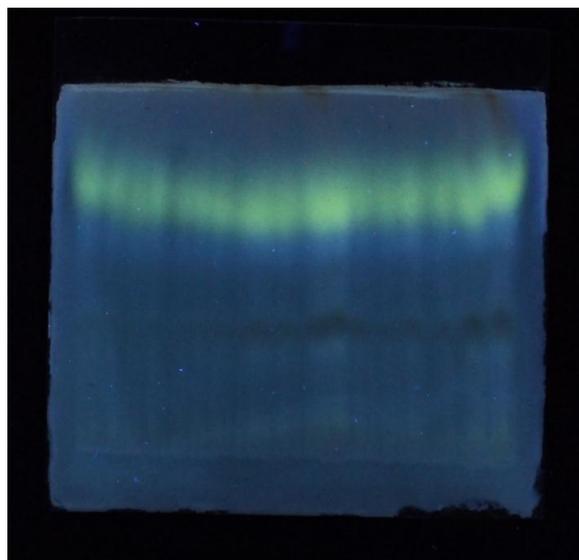


Fig 2: Developed preparative TLC in UV chamber. (UV long wave) Isolated catechin were evaluated by chemical and spectral methods to study the nature of the components. The color characteristics in UV and visible light revealed presence of flavones and/or flavonol in isolated catechin. (Table 3).

Table 11: TLC studies of isolated catechin of extract.

Sr No	Catechin	Rf	Color characteristics	Indication
1	Standard	0.80	Black	Flavones and /or flavonol
2	Isolated	0.80	Dark Blue	Isoflavones

Spectral studies confirmed the results of TLC studies. UV and IR spectra of isolated catechin gave characteristic peaks indicating presence of Flavones. The UV, IR spectra and HPTLC fingerprints of isolated catechin can be used for routine standardization of Cystone tablet (Table 4).

Table 12: Spectral data of isolated fractions

Sr. No	Isolated Catechin	UV	IR (cm ⁻¹)
1	I	245, 254	3412, 3238, 3505, 1144

8. Fingerprint analysis of ethanolic extract of Cystone formulation by HPTLC^[13]

HPTLC fingerprint analysis was carried out on ethanolic extract of Cystone formulation with solvent system i.e. Toluene: Ethyl acetate: Acetone (2:4:4 v/v/v) using Atron HPTLC system consisting of Sparylin spotting, elite-miniluminance photo documentation and CAMAG scanner. The chromatogram obtained was studied under 366 nm (Figure. 3).

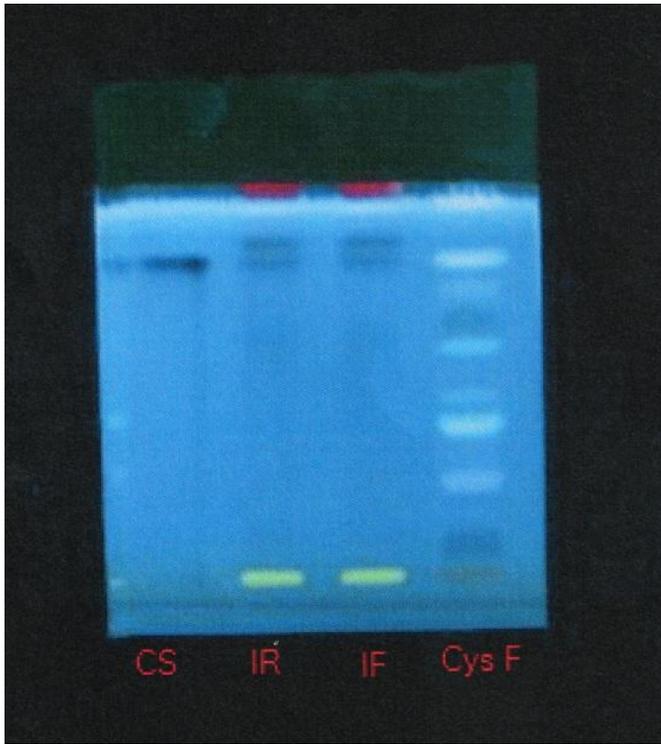


Fig 3: HPTLC fingerprint of Cystone formulation along with marker compounds.

1. CS-Catechin Standard, 2. IR- Isolated from Rhizome of berginia ligulata, 3. IF- Isolated from Cystone formulation, 4. Cys F- Cystone Formulation Extract.

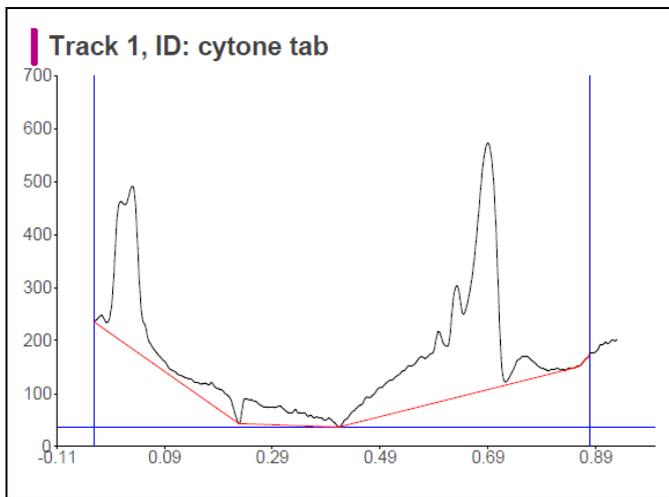


Fig 4: HPTLC chromatogram of Standard Catechin.

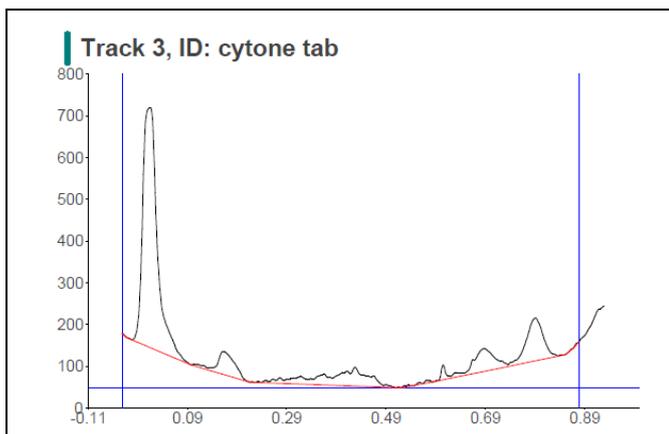


Fig 5: HPTLC chromatogram of Isolated Catechin.

The standard HPTLC chromatogram Standard Catechin and Isolated Catechin showed two peaks with Rf value 0.76 and 0.82. (Figure. 4 and 5)

9. Characterization of extract of Cystone formulation by HPLC

The HPLC analysis of standard Catechin and Isolated Catechin shows peaks with retention times of 2.773 and 2.773 min, respectively, (Figure. 6 and 7).

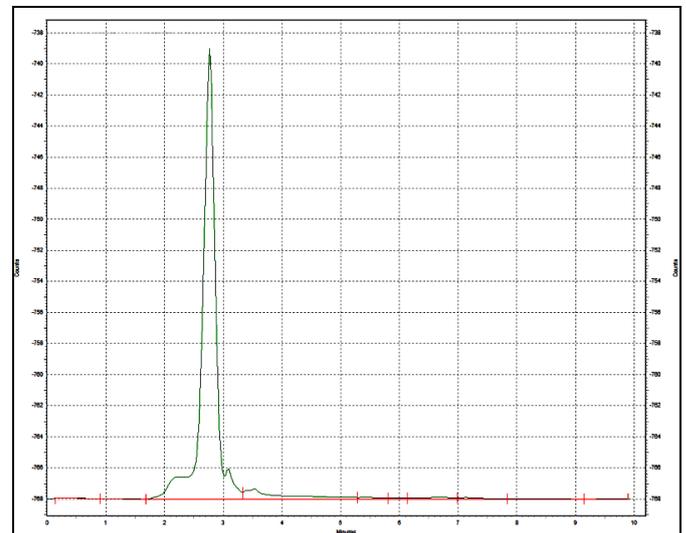


Fig 6: HPLC profile of standard Catechin.

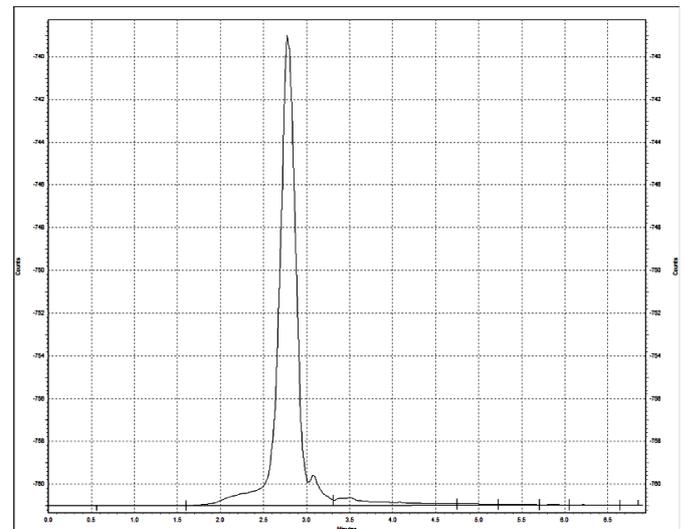


Fig 7: HPLC profile of Isolated Catechin from Formulation.

Thus, it can be said that extract contain catechin. It can be the possible analytical markers for standardization of Cystone formulation. After quantitative estimation of catechin specifications can be stated. This should then serve as a simple, accurate and routine method of analysis for Cystone formulations.

10. Conclusion

The spectral data and HPTLC fingerprint of ethanolic extract of Cystone formulation could be used as a valuable analytical tool in the routine standardization of Cystone formulation to check the batch to bath variation. Catechin can be used as one of the appropriate analytical markers for standardization of Cystone formulation.

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