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A Review on High Performance Thin Layer Chromatography Methods and Validation Parameters for Quantification of Andrographolide from *Andrographis paniculata* and its Marketed Formulations

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Abstract | **Review Article**

Andrographis paniculata is an important medicinal plant belonging to family Acanthaceae and has been used from ancient times. *Andrographis paniculata* is used as a traditional herbal medicine in Unani, Ayurvedic, Homeopathic, and Siddha system. It has potential to treat multiple health diseases like abortifacient, antithrombotic, expectorant, hepatoprotective and other uses also. The major marker chemical constituent found is andrographolide (2.39%) which had many therapeutic uses. Dominancy of the herbal drug depend's on the quantity and quality of therapeutic chemical constituents present in it, which are investigated by using advanced and complex techniques. HPTLC is the advanced and sophisticated technique that can be used for the high-resolution chromatography and identify quality and quantity of therapeutic chemical constituents present in herbal drugs. HPTLC can determine the purity, quality, quantity and authenticity of the herbal crude drugs and marketed formulations very quickly. This article summarised all the relevant information regarding *Andrographis paniculata* and its marketed formulations was collected from various research articles, review articles and availability of *Andrographis paniculata* formulations in the market. Availability of various *Andrographis paniculata* formulations from the market were identified and reported. Literature review on quantification and qualification of marker compound Andrographolide from the *Andrographis paniculata* by HPTLC method with various samples of formulations and whole plant from different locations of India was also done.

Keywords: Andrographis paniculata, High Performance Thin Layer Chromatography, Andrographolide, Hepatoprotective.

1. INTRODUCTION

Herbal medicine often known as phytomedicine, refers to the use of various portions of therapeutic plants. Herbal system of medicine has a long history of use outside of the realm of traditional medicine. It has become mainstream in recent decades as breakthroughs and developments in analysis and quality control, as well as advances in clinical research, have made it possible (HI Raghavendra *et al.*, 2009; Parihar and Sharma, 2021; Telrandhe *et al*., 2021; Chaudhary *et al*., 2021). The practise of prescribing a set of standards or inherent features, consistent parameters, definitive qualitative and quantitate values that convey an assurance of quality, efficacy, safety, and repeatability for herbal medicines is known as standardisation. Standardization is a process that ensures that each packet of medicine sold has the exact amount of active ingredients and will provide the desired therapeutic effects. Standardisation of herbal formulations is necessary for determining the quality of pharmaceuticals based on the concentration of their active principle, physical, chemical standardisation, and invitro and invivo criteria (Kulkarani *et al*., 2019). High Performance Thin Layer Chromatography is an advanced and sophisticated method which may be use for vast range of application. It's far an effective device for high-resolution chromatography. It can trace and evaluate quantitatively the composition of chemical constituents. It's also use for immediate and smooth determination of quality, purity and authenticity of crude drugs and their marketed formulations. Validation is a vital step in knowing that the method used is reliable and reproducible. It is reliable and reliability statistics are especially depend at following conditions, namely, the reliability of the contraptions, proper trained analysts, the methods validity (Modi *et al*., 2016). The parameters as given with the aid of the ICH.

The following validation parameters are commonly monitored for HPTLC approach:

1) SPECIFICITY: - This parameter can be analysing by development and establishment of relation between sample solution and interferences through ingredients present in the formulations. By using standard sample spot values and RF (Retardation Factor) values specificity can be determined (Shewiyo *et al*., 2012).

2) LINEARITY: - Linearity of the method can be analysing by calibration curves. The caliberation curves are constructed at different levels of concentrations. The calibration curves are plotted by taking different ranges of analyte at peak area vs concentrations (Sonia *et al*., 2017).

3) PRECISION: - Precision is of two types inter-day and inter-day precisions. The Intraday precision is calculated through analysing sample solutions at one-of-a-kind three ranges high, low and medium concentrations and calibration curve are made for 5 instances at equal day. Inter-day precision may be calculated with aid of studying sample solutions of three one of a kind ranges this is high, low and medium in a time interval of seven day. Height regions are calculated as %Relative standard deviation (RSD) (Jain *et al*., 2014).

4) Limit of quantifications and detections: - They both are also referred to as sensitivity parameters. LOD is the lowest limit/value of detection at which the drug can be detected or analyse in the sample that is which type of drugs is present in the sample either it is alkaloids containing, terpenoids etc and LOQ is the lowest limit/value of detection at which the drug quantity is detected or analyse that is how much quantity of drug quantity is present in the sample. LOD and LOQ are experimentally confirmed by diluting the recognized concentration (Shankar *et al*., 2020).

5) ROBUSTNESS: - By way of doing small modifications in mobile phase concentrations, as an instance in extent, time of saturation of chamber, and exchange in the distance of migration of solvent. It can be calculated in triplicate and %Relative standard deviation (RSD). Retention factor (Rf) and peak purity are two parameters which is the reason for modifications in chromatographic conditions (Sowganya *et al*., 2015). 6) ACCURACY: - It can be evaluated through studies of 3 levels. Recovery experiment can be executed via including 3 one of a kind quantity of standard drug, i.e. eighty percent, hundred percent, and one hundred twenty percent of the drug, which has to be pre analyzed formulations, and the resultant is reanalyzed 6 instances.

7) Repeatability: - Repeatability or also called as measurement of peak area. It can be calculated by distinctive quantity of analyte with excessive, low and medium degrees. Calibration curve 7 times instances without changing plate position. By spotting samples and taking same range of calibration curves seven times more repeatability can do.

8) Peak Purity: - Three different levels of spectra are compared for peak purity which are: - (a) In the starting point of peak (b) When the peak is at highest position (c) In the last/end of the peak. While doing test on its pure quality, spectra of peak and slope are interlinked with each other. Peak Maximum spectra should taken most of the times with down side slope are use like a referee spectrum for determination. If the test values greater than an error of probability of one percent only then it is invalid or cancelled. It should not be rejected when the values of test and identical are more or 2.576 (Patel *et al*., 2012). Physicochemical properties of 'The Marker Compound Andrographolide' are given in the table 1 (Sareer *et al*., 2014).

Table-1: Physicochemical properties of 'The Marker Compound Andrographolide' (Sareer *et al.*, 2014)

S. No	PHYSICOCHEMICAL PROPERTIES	FEATURES
1	NAME OF MARKER COMPOND	Andrographolide
2	INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY [IUPAC] NAME	3-2-DecaHydro-6-Hydroxy-5-HydroxyMethyl-5,8A-Dimethyl-2-MethyleneNapthyl-Ethylidene-Dihydro-4-HydroxyFuran-2 [3h]-One;
3	FORMULA [MOLECULAR]	$C_{20}H_{30}O_5$
4	CHEMICAL CLASS AND CATOGERY	Labdane DiterpeneLactone; Unsaturated-Tri-HydroxyLactone
5	PHARMACOLOGICAL CATOGERY	PNS [Pheripheral-Nervous-System]
6	WEIGHT [IN MOLECULAR SYSTEM]	350.45 g/m
7	PHYSICOLOGICAL APPEARANCE	Powder form and in solid form
8	TASTE	Bitter
9	COLOUR	Yellowish light and yellowish light brown
10	Odour	No
11	Wavelength max	223 nm
12	Melting point	229-232°C
13	Solubility	Bad solubility in water

S. No	PHYSICOCHEMICAL PROPERTIES	FEATURES
14	Stability	Stable at room temperature
15	Route of administration	Oral route, eyes contact, nasal route
16	Chronic effect on human	Not present
17	Product after combustion	Carbon dioxide, carbon monoxide, carbon oxides
18	Burnable and incendiary	Flammable on increasing temp.
19	Storage	To be kept in cool and dry place and well-ventilated place

2. USES OF *ANDROGRAPHIS PANICULATA*

Antiviral, anti-alzhemier (Rivera *et al*., 2016), antioedema (Lin *et al*., 2019), antipyretic, analgesic (Suebsasana *et al.,* 2009), antifungal (Yadav *et al*.,2012), antimicrobial (Singha *et al*., 2003; Soumya *et al*., 2014; Tanwar *et al.,* 2016), antibacterial (Malahubban *et al*., 2013; Mandal *et al*., 2016), antioxidant (Rafar *et al.,* 2010; Vasu *et al*., 2010), anticancer (Shi *et al*., 2008, Lee *et al*.,2010, Nadiminty *et al*., 2010; Zhou *et al.,* 2010; Yue *et al*., 2015; Tan *et al.,* 2016; Yuan *et al.,* 2016; Wang *et al*., 2016), anti-inflammatory (Shen *et al*., 2002, Chao *et al*., 20019; Zou *et al*., 2016), hepatoprotective (Rammohan *et al*., 2008; Zhang *et al*., 2000), antihyperglycemic (Yu *et al.,* 2003), platelet activation (Lu *et al*., 2011), antiulcerogenic (Madhav *et al*., 1995). TABLE 2 showed Marketed formulations of *Andrographis paniculata* according to AYUSH.

Table-2: Marketed formulations of *Andrographis paniculata* according to AYUSH (Ayurvedic Pharmacopoeia, Siddha Pharmacopoeia, Unani Pharmacopoeia, Homeopathic Pharmacopoeia)

AYURVEDIC FORMULATIONS	SIDDHA FORMULATIONS	HOMEOPATHY FORMULATIONS	UNANI FORMULATIONS
• Kalmegh (60 capsules) • Vasu vasuliv tablets • Liv d 38 tablets • Organic india kalmegh capsules • DBS Kalmegh syrup • Garlico herbal kalmegh powder	• SKM [Siddha and ayurvedha Madhumegha Kudineer Chooranam] • Avarai Kuduneer Churanam • Kabasura kudineer • Nilavembu kudineer chroonam • Vatha sura kudineer chroonam • Kabasura kudineer chroonam	• Dr.Reckeweg Kalmegh Tinture Q • Bakson kalmegh aid • SBL Homeopathic kalmegh drops (30ml) • SBL Andrographis paniculata mother tincture Q • Wheezal kalmegh drops • Dr. Willmar Schwabe Andrographis paniculata Q • Syrup kalmegh homeopathic medicine,200 ml Liquids	• Arq-e-Chiraita • Majoon-e-Chiraita • Laooq-e-Chiraita • Jawarish-e-Chiraita • Khamira-e-Chiraita

3. High performance thin layer chromatography methods and validation parameters for quantification of andrographolide from *andrographis paniculata* and its marketed formulations.

Bhope *et al*. in 2009 had reported a new and precise method from HPTLC from quantitative estimation Marker Compound [Andrographolide] in Andrographis paniculata and marketed formulations. The stationary phase used was precoated [silica gel 60 F254] Aluminium plates. The mobile phase used was Methanol (2.5); Formic Acid (05); Ethyl Acetate (30): Toulene (50). The retention factor for andrographolide was found to be 0.34 and plus minus 0.03 in both case i.e pharmaceutical dosage and plant extract. The linearity was found at 100-800 ng per spot. The method is precise by relative standard deviation value was 1.52 percent and relative standard deviation for system precision was 1.38 percent. The accuracy was 97.34 plus minus 1.47 and specificity was according to ICH guidelines.

Jadhao in 2010 had reported rapid, new and accurate method for determination of marker compound andrographolide in polyherbal formulations or herbal powder from Andrographis paniculata by HPTLC method. The method contains isolation of compounds by thin layer chromatography method plates in which were precoated with silical gel 60F 254. The solvent system consists of benzene: ethyl acetate 5:5 [v/v]. The scanning was done through densitometric scanner (UV reflectance photomode) at 220 nano metre. The amount of andrographolide content found in test sample was 237.2 gram in per 100 mg herbal powder and 41.80 mg in per 5 ml of polyherbal formulation. The linearity was 360 ng to 660 ng per spot and the average % recovery was 97.68.

Mamatha in 2010 had reported and validated HPTLC method in which Andrographolide is estimated quantitatively in *A. paniculata*. The technique hired TLC Aluminum (Al) precoated silica gel 60 F254 which plays are role of stationary segment with mobile

segment as Chloroform: Methanol 7:1v/v. Andrographolide confirmed suggest Rf value is 0.41 with λ max at 231nm. The approach turned into verified in phrases of linearity (100– 500ng), precision, and accuracy (100.03% restoration). Limit of detection became 30ng and restrict of quantification 100ng. Inter day variation 1.14% -1.25%, intraday variation 1.14% - 1.58%.

Jain *et al*. in 2010 had reported a new method for quantitative estimation of marker compound from Andrographis paniculata extract and its marketed formulations. The separation for active drug has done by chromatography on the pre-coated (silica gel 60 F254) aluminum plates which act as a stationary phase. The solvent system contains chloroform: toluene: methanol 66:26:08[v/v/v]. The retention factor of andrographolide was 0.49. The wavelength used for analysis was 229 nano meters. The linearity range was found to be 200 to 1000 ng, limit of detection was 3.5ng and limit of quantification was 11.7 ng.

Patel *et al.* in 2012 had reported HPTLC method to quantify marker compound andrographolide in tablet and syrup formulations of *Andrographis paniculata.* Stationary Phase was Precoated silica 60F254 plates and Mobile Phase is toluene: acetone: formic acid (9:6:1) and detected wavelength value 254 nanometer had been used. LOD or LOQ of andrographolide was 79.28ng/spot or 26.16ng/spot. Inter day (n=5) 0.63, Intraday (n=5) zero 0.36. The calibration curve was linear among 200-400 nano gram per spot for marker compound and a 100 -200nanogram per spot.

Zade *et al*. in 2013 had reported new, simple and precise method of validation and quantitative estimation of Andrographis paniculata and its marketed formulations (Amylcure, Livomyn, Liv-compound) through HPTLC. The solvent system used is Methanol: Chloroform in ratio 1:9v/v and stationary phase used was aluminiun plate 60 F254. Wavelength used for scanning andrographolide was 232 nanometer. Retention factor value for andrographolide is 0.64. Limit of detection and limit of quantification for andrographolide was 3.05 ng/spot and 18.28 ng/spot. Linearity range was 200-1000 ng/spot for andrographolide.

Garg *et al*. in 2016 had validated balance indicating research of kalmegh by HPTLC. Stationary phase used was silica gel 60F254, cell section used chloroform: toluene: methanol (7:1.5:1.5, v/v/v), wavelength range was 254 nm. Limit of quantification was 0.52ng/spot and limit of detection was 1.59 ng/spot. Linear variety 100-500 ng/spot, %RSD (n=6) 0.8686-1.74, Intraday -zero.56-1.74, Interday 67.392-128.63. Linear regression equation was Y= mx +c Y= 3.534x +3510.

Pancham *et al.* in 2019 had reported a validated an appropriate analytical technique for the Andrographolide amount determination in its bulk powder. Ultra Voilet-Spectrophotometric approach had developed. Solvent used was methanol: water (50:50v/v). The wavelength used is 321nanometer. Restriction of detection 14.71ug/ml, Limit of quantification LOQ is 44.57 ug/ml, interaday version 0.868 %– 1.203 %, machine Precision is 1.8% Inter-day one was 1.51%, Inter day two was 1.90%, Inter day three was 1.64%. Analyte confirmed linear reaction between the attention varieties of 50-250µg/mL. % Relative Standard Deviations values less than two percent. An approach may be done on an excellent manage and test of andrographolide/marker compound in bulk powder of *Andrographis paniculata*.

4. High-performance thin layer chromatography methods and validation parameters for quantification of other than andrographolide from *andrographis paniculata* and its marketed formulations.

Akowuah *et al*. in 2009 had reported a new simple and rapid method for quantitative determination of 14—Deoxy-11, 12-DidehydroAndrographolide and Andrographolide with HPTLC method and RP-HPLC with UV method. The RSD i.e relative standard deviation of both the marker compound was found between range 0.86--0.99 for intra-day and 0.86-0.98 for inter-day from HPTLC method. The RSD i.e relative standard deviation of andrographolide and 14-deoxy-11, 12-didehydroandrographolide was in between the range of 0.86-1.02 for intra-day and 0.87-1.02 for inter-day from HPLC method. Recovery range of andrographolide and 14-deoxy-11, 12-didehydroandrographolide was 96.5 to 99.0 percent from HPTLC. The leaves used were of plants cultivated in different locations of Malaysia. Both fingerprinting techniques showed similar quantity of in andrographolide and 14-deoxy-11, 12-didehydroandrographolide all extract.

Pawar *et al*. in 2010 had reported rapid and accurate HPTLC method for determination of andrographolide into variety of samples in whole part of plant *Andrographis paniculata.* The separation of andrographonin was done by putting spot of alcholic extract of the whole plant i.e *Andrographis paniculata.* Stationary phase used was pre-coated (silica gel 60 GF254) aluminum plates. The solvent system used was Formic acid (0.5); Ethyl Acetate (4.5) Toulene(5.4). The derivatizing is done by using anisaldehyde – sulphuric acid reagent. The wavelength used for detection and quantitative analysis of compounds was 235 nano meter. The samples contain same amount of marker compound. The mean recovery was nearly 100% and found to be accurate. TABLE 3 showed Validation parameters of andrographolide content form *Andrographis paniculata* by HPTLC from different geographical areas.

Table-3: Validation parameters of andrographolide content form *Andrographis paniculata* by HPTLC from different geographical areas

Herbal extracts \formulations of *ANDROGRAPHIS PANICULATA*	Amount of andrographoloide present	RF	LOD (limit of detection)	LOQ (limit of quantification)	RSD %	%Recovery	Absorbance wavelenght	Mobile phase	References
Madhya Pradesh (leaves of kalmegh)	2.32%	0.49	3.5 ng	11.7 ng	0.0164	99.70±0.254	229nm	Chloroform:toluene:methanol (66:26:8,v/v/v)	Jain *et al*.,2010
Maharastra (leaves of kalmegh)	2.37%	0.49	3.5 ng	11.7 ng	0.0137	98.9±1.569	229 nm	Chloroform:toluene:methanol (66:26:8,v/v/v)	Jain *et al*.,2010
Uttar Pradesh (leaves of kalmegh)	2.46%	0.49	3.5 ng	11.7 ng	0.0113	97.8±1.065	229 nm	Chloroform:toluene:methanol (66:26:8,v/v/v)	Jain *et al*.,2010
Karnataka (kalmegh leaves)	1.19%	0.41	30 ng	100 ng	1.19%	4.864+9.11*X	231 nm	Chloroform:Methanol (7:1)	Mamatha 2010
Tamil Nadu (Kalmegh leaves)	0.85%	0.41	30 ng	100 ng	1.19%	4.864+9.11*X	231 nm	Chloroform:Methanol (7:1)	Mamatha 2010
Andhra Pradesh (Kalmegh leaves)	0.70%	0.41	30 ng	100 ng	1.19%	4.864+9.11*X	231 nm	Chloroform:Methanol (7:1)	Mamatha 2010
Kerala (Kalmegh leaves)	0.99%	0.41	30 ng	100 ng	1.19%	4.864+9.11*X	231 nm	Chloroform:Methanol (7:1)	Mamatha 2010
Bangalore *Andrographis paniculata* (AP) whole plant	97.34%	0.34 ± 0.03	30 ng	100 ng	Method precision =1.52 %, system precision = 1.38%	y=142.24 x + 138.05	226 nm	Methanol (2.5): formicacid(05):ethyl acetate(30):toulene(50)	Bhope *et al*., 2009

The amount of marker compound andrographolide varies from place to place, which results in variations in the formulations also. The HPTLC method validation can resolve the quality and quantity of marker compound andrographolide.

There are different geographical conditions of different geographical areas, so the concentration of the chief constituent's andrographolide also varies. It also results in variations of marker compound in formulations.

The variations in the amount of andrographolide are due to different soil physiology and pH range and different climatic condition of each and every place.

To know the variations in amount of andrographolide the biosynthetic pathways were also studied. And it came to know that the enzymes, coenzymes and precursor compounds in between biosynthetic pathways has different pH and temperature range which affect the quantity of andrographolide in different geographic conditions. pH range and climatic temperature of soils and precursor compounds of andrographolide are given in table 4.2 and 4.3 respectively. TABLE 4 showed pH range and climatic temperature of soils of different areas (S.K. Reza *et al*., 2017). TABLE 5 showed pH range and climatic temperature of precursor compounds of andrographolide. TABLE 6 showed Validation parameters of andrographolide content form *Andrographis paniculata* by HPTLC from different marketed formulations. TABLE 7 showed HPTLC for other than andrographolide content from marketed formulations.

Table-4: pH range and climatic temperature of soils of different areas (S.K. Reza *et al*., 2017)

S. NO	NAME OF PLACE	pH RANGE	CLIMATIC TEMPERATURE	REFERENCES
1	Karnataka	3.9-7	Hot with heavy rainfall	http://saspublisher.com/wp-content/uploads/2013/10/SAJB-15200-208.pdf
2	Kerela	3.5-5.5	28 -32degree c	https://www.keralasoilfertility.net/en/laterites.jsp#:~:text=More%20than%2090%20per%20cent,with%20lime%20to%20alleviate%20acidity
3	Andhra Pradesh	6.5-8	Hot and humid	https://andhrapradesh.pscnotes.com/andhra-general-studies/soils-of-andhra pradesh/
4	Madhya Pradesh	5.5-8.5	Subtropical climate	https://madhyapradesh.pscnotes.com/madhya-pradesh-gk/madhya-pradesh geography/soils-of-madhya pradesh/#:~:text=The%20pH%20level%20of%20these,%2C%20Sidhi%2C%20Katni%2C%20Umaria.&text=This%20type%20of%20soils%20is,matter%20and%2 0Phosphorous%20is%20less

S. NO	NAME OF PLACE	pH RANGE	CLIMATIC TEMPERATURE	REFERENCES
5	Uttar Pradesh	6.9-9.5	Fluctuating from 0-32 degree C	https://www.researchgate.net/figure/Soil-pH-status-in-four-selected-districts-of eastern-Uttar-Pradesh_tbl1_283203171
6	Bangalore	4.6-5	28 – 32 degree C	https://www.researchgate.net/figure/Soil-pH-CaCl2-in-ab-clay-loam-BCL-red loam-RL-and-brown-sandy-loam-BSL-soil_fig4_299575399

Table-5: pH range and climatic temperature of precursor compounds of andrographolide

S.NO	PRECURSORS NAME	pH RANGE	TEMPERATURE	REFERENCES
1	IPP [Isopenteyl Pyrophosphate]	7-7.8	4 degree C	Valdiva *et al*. 1997
2	DMAPP [Dimethylalallyl pyrophosphate].	7	Not mention	Takahashi *et al*. 1999
3	DXP [Deoxyxylulose]	5.5-9.0	Not mention	Bailey *et al*. 2002
4	MVA [Mevalonic acid] pathways.	7-10	Not mention	Schulte *et al*. 2000
5	HMGO coenzyme A	7.2	Not mention	Takahashi *et al*. 1999
6	Acetyl coenzyme A	4.7-9.0	Not mention	Walker *et al*. 1999

Table-6: Validation parameters of andrographolide content form *Andrographis paniculata* by HPTLC from different marketed formulations

Herbal extracts \formulations of *ANDROGRAPHIS PANICULATA*	Amount of andrographoloide present	RF	LOD (limit of detection)	LOQ (limit of quantification)	RSD %	%Recovery	Absorbance wavelenght	Mobile phase	Refrence
Formulation 1 (20 tablets, 600mg wt.)	1.28%	0.49	3.5 ng	11.7 ng	0.0135	96.8±1.05	229 nm	Chloroform:toluene:methanol (66:26:8,v/v/v)	Jain *et al*.,2010
Formulation 2 (20 tablets, 600mg wt.)	1.68%	0.49	3.5 ng	11.7 ng	0.0175	98.0±0.75	229 nm	Chloroform:toluene:methanol (66:26:8,v/v/v)	Jain *et al*.,2010
Formulation 3 (20 tablets, 600mg wt.)	1.41%	0.49	3.5 ng	11.7 ng	0.0261	98.5±2.61	229 nm	Chloroform:toluene:methanol (66:26:8,v/v/v)	Jain *et al*.,2010
Formulation 4 (20 tablets, 600mg wt.)	1.05%	0.49	3.5 ng	11.7 ng	0.0273	98.96±1.66	229 nm	Chloroform:toluene:methanol (66:26:8,v/v/v)	Jain *et al*.,2010
olyherbal Asava (Kalmegh)	41.80 mg/ 5ml	0.10			1.080	97.68%	220 nm	Benzene:ethyl acetate (5:5)	Jadhao 2010
Herbal powder (Kalmegh)	237.2 ug/100 mg	0.10			1.080	97.68%	220 nm	Benzene:ethyl acetate (5:5)	Jadhao 2010
Amylcure (kalmegh marketed formulation)	585.24 ng	0.64	3.05	18.29	0.551	99.13±0.16	232 nm	Chloroform:Methanol [9:1]	Zade *et al*. 2013
Livomyn (kalmegh marketed formulation)	175.23 ng	0.64	3.05	18.29	0.501	99.45±0.17	232 nm	Chloroform:Methanol [9:1]	Zade *et al*. 2013
Liv-Compund (kalmegh marketed formulation)	407.14 ng	0.64	3.05	18.29	0.588	99.32±0.16	232 nm	Chloroform:Methanol [9:1]	Zade *et al*. 2013

Table-7: HPTLC for other than andrographolide content from marketed formulations

Marketed formulation	Chemical constituents	LOD	LOQ	RF value	Mobile Phase	Wavelenght	References
Navayas Loha (Ayurvedic formulation)	Andrographonin	99.60 %	0.05194 % w/w	0.57	Toulene:Ethyleacetate:Formic acid (5:4:1)	235 nm	Pawar *et. al*., 2011

There was a large amount of variations in amount of andrographolide in different formulations of *Andrographis paniculata.* But the validation and HPTLC method was able to adequately resolve the standard and quantify it. TABLE 8 showed Validation by HPTLC for other than andrographolide content from crude drug

Table-8: Validation by HPTLC for other than andrographolide content from crude drug

Sample	Chemical constituents	%Recovery	Quantity presents	LOD	RSD	RF	Mobile phase	Absorbance wavelenght	Refrence
Kalmegh	14-deoxy-11, 12-didehydroandrographolide	96.5–99.0%	21.7-26.9 mg/g	3.6 ug/ml	1.47 %	0.43	Chloroform:methanol (8:2)	254 nm	Akhowa *et al*., 2009
Kalmegh [whole plant]	Andrographonin	99.82%	0.04966 % w/w			0.57	Toulene:Ethyleacetate :Formic acid (5:4:1)	235 nm	Pawar *et al*., 2011

Validation and HPTLC of other then marker compound was also done. The name of the chemical constituents is 14—Deoxy-11, 12-DidehydroAndrographolide and Andrographonin which is also present in *Andrographis paniculata.* The limit of detection for 14-deoxy-11, 12-didehydroandrographolide is 3.6 ug/ml and quantity of 14-deoxy-11, 12-didehydroandrographolide present is 21.7-26.9 mg/g.

5. CONCLUSION

In the present work ‘’to review high performance thin layer chromatography methods and validation parameters for quantification of andrographolide from *andrographis paniculata* and its marketed formulations**’’,** all the relevant information regarding *Andrographis paniculata* and its marketed formulations was collected from various research articles, review articles and availability of *Andrographis paniculata* formulations in the market. Quality and quantity of *Andrographis paniculata* varies due to the different geographical conditions of different geographical areas. So, the concentration of the chief constituent andrographolide also varies. It also results in variations of marker compound in formulations. The variations in the amount of andrographolide are due to different soil physiology and pH range and different climatic condition of each and every place. To know the variations in amount of andrographolide the biosynthetic pathways were also studied. And it came to know that the enzymes, coenzymes and precursor compounds in between biosynthetic pathways has different pH and temperature range which affect the quantity of andrographolide in different geographic conditions. So, there is a need to maintain its quality.

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