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A Review of Markers in Ayurveda for Anti-Inflammatory Drugs

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Abstract

From the ancient days itself the ayurvedic medicine has special importance. This ayurvedic system of medicine originated in India. In India nearly 70% of people use ayurvedic system of medicine along the western system of medicine. The Ayurveda mainly come under three classes are vata, pitta and the kapha. Chemically identified constituents of an herbal medicine that are of concern for quality control purposes are known as markers. Some of the markers are widely used for anti-inflammatory agents are *Boswellia, sunthi, gokshuru, guduchi, guggulu* this marker possess anti-inflammatory action that reduces pain, swelling of joints, Arthritis, joint complaints, Muscular injuries etc. The chromatographical / spectroscopical analytical development can be done for markers. The analytical development possess the way to the analytical information about the markers. The technique such as HPLC, HPTLC, LC-MS, UPLC-PDA are properly validated by ICH guidelines using validation parameters such as accuracy, recovery, precision, LOD, LOQ, Robustness etc. For many markers there is lack of development of Hyphenated techniques. By increasing the analytical techniques, research is prone to be more effective and successful to promote quality, safety and efficacy.

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INTRODUCTION

Anti-inflammatory drugs

Anti-inflammatory drugs are a form of medication that relieves pain, lowers fever, prevents blood clots, and reduces inflammation in higher doses.

Role of Markers in Herbal Medicines

The World Health Organization divides herbal medicines into three categories: raw plant material, processed organic matter, and medicinal, nutritional supplements. Herbal drugs are labelled finished products that contain active compounds such as aerial or underground parts of the plant, other plant material, or a combination of them, whether in their natural state or as plant preparations. Herbal drug use has sky rocketed in recent years, following a worldwide pattern of people turning to natural therapies. People take herbal medicine items, which come in the form of tablets, capsules, powders, teas, oils, and fresh or dried plants, to improve their health. Proper identification and quality assurance of the starting material are needed to ensure reproducible quality of herbal medicine, which contributes to its safety, quality and efficacy.

Factors influencing identification and quality of herbal drugs

1. Plants are typically made up of a number of constituents.

2. The raw material's source and consistency are unreliable.

3. In some situations, the active concept is unclear.

4. It's *possible* that specialised analytical techniques or reference compounds aren't commercially available.

5. Chemically and naturally, plant materials vary.

6. The appropriate herb is intentionally or unintentionally substituted with other low-quality and morphologically similar medicinal or non-medicinal plants, such as Belladonna leaves for Ailanthus leaves, papaya seeds for Piper nigrum L.

7. Collecting, drying, storage, transportation, and processing methods have an impact; for example, Datura stramonium L. leaves should be collected during the flowering stage, and wild cherry bark should be collected in the autumn.

8. Different vernacular names for different plants for the same medicinal plant in different states. Centella Asiatica L. and Bacopa monnerri L., for example, are both referred to as Brahmi and Mandukparni.

Markers

A marker component is a naturally occurring component in a material that has been chosen for special attention (for identification or standardisation purposes).

or

Identifiers are scientifically identified components of herbal medicines that are used for quality control purposes, whether or not they have antimicrobial effects. Markers could be used to determine the amount of active ingredient in the final result of a herbal remedy or preparation.

Markers widely used as Anti-Inflammatory drugs in the Ayurvedic form

The widely used markers are

1. Boswellia Serrata -Shallaki

2. Sunthi - Zinger officinalae

3. Gokshura -Tribulus terrestris

4. Guduchi - Tinospora cordifolia

5. Guggulu -Commiphora wightii

Boswellia Serrata

The herb *Boswellia serrata*, also known as Shallaki, is commonly used in Ayurveda for joint support and overall well-being. Boswellia serrata gum resin was used in ancient and African folk medicine to treat arthritis. Boswellia greatly decreases glycosamide degradation and protects joints.

It has a wide range of health and immune-modulating properties, as well as anti-inflammatory and anti-atherosclerotic properties. It also increases blood supply and restores the integrity of blood vessels that have been damaged by spasms. It's also used in cosmetics, such as skin health gummies. Boswellic acid is a marker compound used in Boswellia serrata gum resins. (Ammon, 2016). Alpha and beta Boswellic acid (shown in Figure 1 and Figure 2)

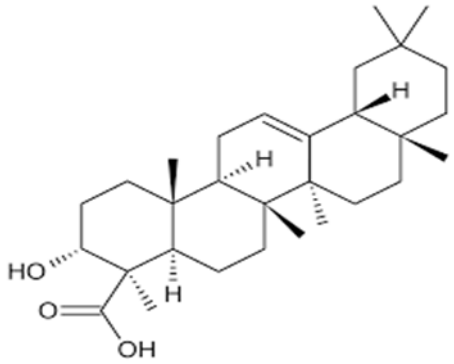

Figure 1: Alpha Boswellic acid

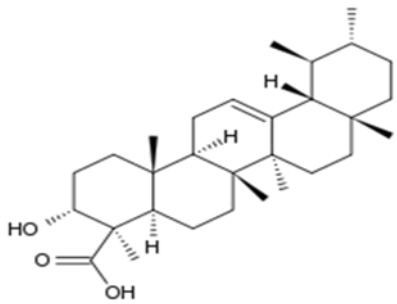

Figure 2: Beta Boswellic acid

Chemical Marker

The organic acids boswellic acids are made up of a penta cyclic triterpine, carboxylic group, and other functional groups. Both alpha and beta boswellic acid (C30H48O3) have an extra hydroxyl group; the only difference is the triterpine structure.

The oleo gum resins contain 30 to 60% resins, 5 to 10% essential oil, 65% arabinose, galactose, xylose.

Types of Chemical Marker

Active principle

Chemicals with well-defined clinical activity.

Example: Alpha boswellic acid and beta boswellic acid is present in boswellia serrata

Active marker

They are substances or groups of substances that have been shown to have pharmacological activity. This helps with effectiveness.Ex.C30H48O3 used for anti-inflammatory action. (Engels, 2010)

Analytical marker

They are constituents or groups of constituents that help in the positive identification of raw materials and extracts are used to achieve standardisation and validity in analytical purposes.

The inclusion of boswellic acid is safe.

Analytical development

Several analytical techniques for the analysis of Boswellic acid have been published in this article, including UPLC-PDA, TLC, and LC-MS.

Method validation

The procedure has been tested in accordance with ICH guidelines. Method validation is performed to ensure that the study is sufficient for its intended intent.

Chromatographic methods were referred to by several reverse phase columns of HPTLC. Such as acquity DHC18[100mm*2.1mm], phenyl – hexyl [100mm *2.1mm] and hypersil gold C18[150mm*2.1mm] to achieve a short run time, symmetric peak shape and better chromatographic efficiency.

There are six references. Keto boswellic acid, 3-O-acetyl-11-keto beta boswellic acid, alpha boswellic acid 3-O-acety, beta boswellic acid, 3-0-acetyl-a-boswellic acid, and 3-o-acetyl-b-boswellic acid are examples of boswellic acids. Linearity and regression are quantified using UPLC-PDA methods and validated using validation parameters that meet ICH guidelines. Retention time, Linearity, Regression equation for six boswellic acids (shown in Table 1). R^2, LOD, LOQ, %RSD of six Boswellic acids (shown in Table 2).

Electro spray Ionization time of light mass spectrometer [ESI-MS] and ultra-high-performance liquid chromatography diode array deduction For metabolite recognition and elucidation. Fragmentation of boswellia serrata for secondary metabolite characterization using UPLC/PDA value (shown in Table 3) Via mass fragmentation in LC-MS, B.serrata or tri terpenoids belonging to boswellic acid and Tirucallic acid tri metabolites were discovered. Retention time, λ max of LC-MS of six Boswellic Acid (shown in Table 4).

Sunthi

Sunthi, also known as Ginger or Zingiber officinale, is a popular dietary supplement, spice, and flavouring agent in food and beverages. It is used in treatment such as arthritis, rheumatism, sprains, muscular discomfort, infectious diseases, and helminthiasis in the Ayurvedic system of medicine. Ginger contains a variety of pungent and biologically active components, including Gingerol. Structure of gingerol (shown in Figure 3)

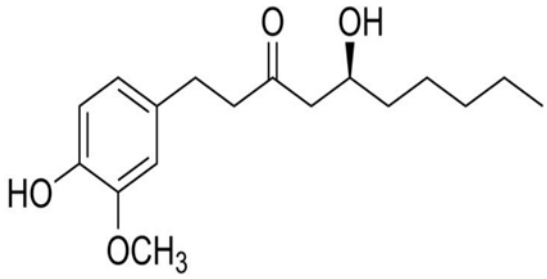

Figure 3: Structure of Gingerol

Chemical Markers

Ginger contains a number of biologically active component such as 6-Gingerol, 8-Gingerol, Zingerone and 6-Paradol. The aromatic component includes Zingiberene and Bisabolene, while the Pungent component includes Gingerols and shogaols. (Govintharajan, 1982)

Types of Chemical Markers

Active principle

Chemicals with well-defined clinical activity.

Example: Gingerol in ginger.

Active marker

They are pharmacologically active constituents or classes of constituents that contribute to efficacy.

Example: 8-Gingerol, 6-Gingerol in anti-inflammation, (Mustafa and Srivastava, 1990) anti-platelet aggregation activities etc.

Analytical marker

They are a constituent or a group of constituents that help in the positive identification of raw materials and are used to achieve standardization and validation.

Analytical development

Several analytical techniques for the analysis of Gingerols in ginger have been published in this article, including HPLC, GC-MS, and LC-MS.

Method validation

The procedure has been tested in accordance with ICH guidelines. Method validation is performed to ensure that the study is sufficient for its intended intent.

Table 1: Retention time, Linearity, Regression equation for six Boswellic acids

S. No.	Analyte	Retention time (mins)	Linearity	Regression equation
1.	Ketoboswellic acid	2.95	1-500	Y=18138X-17092
2.	3-O-Acetyll 11-keto B-boswellic acid	5.66	1-500	Y=20645X-49509
3.	α-Boswellic acid	7.89	1-500	Y=6705X-14231
4.	β – Boswellic acid	8.35	1-500	Y=3251X-6391
5.	3-O-Acetyl α-boswellic acid	11.72	1-500	Y=4942X-13251
6.	3-O-Acetyl β-boswellic acid	12.22	1-500	Y=9085X-23136

Table 2: R^2, LOD, LOQ, %RSD of six Boswellic acid

S. No.	Analyte	R2	LOD	LOQ	%RSD
1.	Ketoboswellic acid	0.999	0.10	0.50	0.44
2.	3-O-Acetyll 11-keto B-boswellic acid	0.999	0.10	0.50	1.26
3.	α-Boswellic acid	0.999	0.50	1.00	3.72
4.	β – Boswellic acid	0.999	0.50	1.00	2.61
5.	3-O-Acetyl α-boswellic acid	0.999	0.40	1.00	3.42
6.	3-O-Acetyl β-boswellic acid	0.999	0.40	1.00	1.28

Table 3: UPLC – PDA values

S. No.	Analyte	UPLC – PDA Value
1.	Ketoboswellic acid	0.94 ± 0.02
2.	3-O-Acetyll 11-keto B-boswellic acid	0.26 ± 0.01
3.	α-Boswellic acid	0.28 ± 0.01
4.	β – Boswellic acid	1.22 ± 0.05
5.	3-O-Acetyl α-boswellic acid	0.61 ± 0.02
6.	3-O-Acetyl β-boswellic acid	0.25 ± 0.01

Table 4: Retention time, λ max of LC-MS of six Boswellic Acid

S. No.	Analyte	Retention time	λ max (nm)
1.	Ketoboswellic acid	3.20	250.42
2.	3-O-Acetyll 11-keto B-boswellic acid	6.13	228.42
3.	α-Boswellic acid	8.41	225.42
4.	β – Boswellic acid	8.91	225.42
5.	3-O-Acetyl α-boswellic acid	12.30	226.42
6.	3-O-Acetyl β-boswellic acid	12.79	226.42

The HPTLC system made up of linomat IV, CAMAG TLC scanner. The TLC was optimized using different solids of varying polarity is used for the identification of secondary metabolites. 1st, 2nd, 3^{rd}, measurement and linear regression curve of 6 and 8 gingerols in TLC is (shown in Table 5).

Within one laboratory, each measurement was carried out by a different Analyte on different days using HPTLC plates.

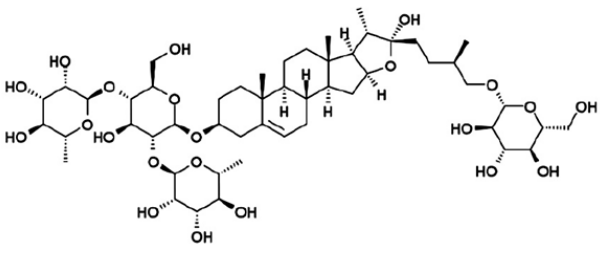

Figure 4: Structure of protodioscin

Table 5: 1^{st}, 2^{nd}, 3^{rd}, measurement and linear regression curve of 6 and 8 gingerol in TLC

Analyte	1^{st} measurement	2^{nd} measurement	3^{rd} measurement	Linear regression curve
6-Gingerol	0.62	0.14	0.50	Y= - 913.1+18.73X
8-Gingerol	4.85	2.95	2.65	Y= -313.8+15.20X

Table 6: HPLC – 6-Gingerol

Nominal concentration	Measured concentration	S. D.	CV (%)
85.0	85.94 ± 0.39	0.87	1.01
6.5	6.73 ± 0.01	0.01	0.16
5.0	5.52 ± 0.06	0.14	2.44

Table 7: Retention time of 6 and 8 gingerol in LC-MS

Analyte	Retention time
6-Gingerol	4.12
8-Gingerol	5.25

The extracts were analyzed on an HPLC system consisting of a shimadzu LC 20-A prominence controller, photo diode array deductor (Schzetner and Rios, 2007). HPLC of 6-gingerol is (shown in Table 6). The identification of secondary metabolites through the spectra. The method is validated on the suitable parameters Accuracy, precision, repeatability, LOD, LOQ.

For the determination of 6, 8 gingerol, a rapid and sensitive ultra-performance liquid chromatography-tandem mass spectrometry method was developed and validated. The chromatographic separation achieved isocratically on C_{18}column. Mass spectrometry transition for 6, 8 gingerol transition occurred at m/z ratio. The retention time of 6 and 8 gingerol in LC-MS (shown in Table 7).

Gokshura

Gokshura, also known as *Tribulus Terrestris* as long been used in oriental ayurvedic medicine for the usage of diuretic, anthelmintic, treating cough, kidney failure. This medical plant is also used in various ayurvedic formulation in combination with other medicinal plants in the treatment of osteoarthritis. An important component in the Gokshura is protodioscin. Structure of protodioscin (shown in Figure 4).

Chemical Marker

Many active ingredients are found in Tribulus Terrestris, including steroidal saponins such as diosgenin and protodioscin. 2014 (Hostettmann, 2014) Tribulus also contains phytosterols, especially beta sitosterols, which are beneficial to the prostate, urinary, and cardiovascular systems.

Types of Chemical Marker

Active principle

Chemicals with well-defined clinical activity.

Example: Protodioscin in Tribulus Terrestris

Active marker

They are pharmacologically active constituents or classes of constituents that contribute to efficacy.

Example: Diosgenin and protodioscin in osteo arthritis, urinary aliments.

Analytical marker

They are a constituent or a group of constituents that help in the positive identification of raw materials and are used to achieve standardisation and validation.

Analytical development

In this study, several analytical techniques have been reported for the analysis of protodioscin in Gokshura, like HPLC, HPTLC.

Method validation

The procedure has been tested in accordance with ICH guidelines. Method validation is performed to ensure that the study is sufficient for its intended intent.

UV spectra were scanned using instrument lambda, and chromatography was performed on an HPLC system with a binary pump diode array deductor. (Gupta *et al.*, 2012) The separation was per-

formed on HPLC column RP-18E coupled with guard column using UV deduction for identification of secondary metabolites. Retention time, Correlation Coefficient of HPLC for Tribulus Terrestris (shown in Table 8).

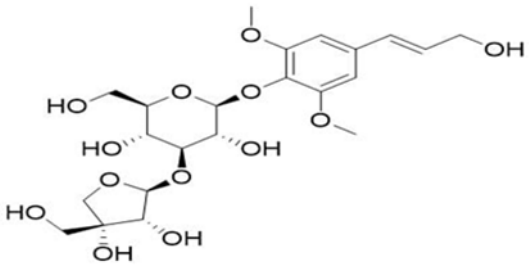

Figure 5: Structure of Cordiofoloside

For the simultaneous quantification of protodioscin and prototribestin, a simple and repeatable HPTLC method was developed (Alam *et al.*, 2016) using WINCAT software. HPTLC analysis is a strong recognition method. HPTLC is an instrumental technique that uses special plates and instruments for sampling and separation, with densitometry assisting the process. After that, the established method was tested for specificity, precision, linearity, LOD, LOQ, and recovery. Rf, Regression equation, R^2, LOD, LOQ data of Diosgenin, Protodioscin (shown in Table 9).

Guduchi

Guduchi is also known as *Tinospora cordifolia*in, the ayurvedic system of medicine guduchi is widely used in the treatment of jaundice, rheumatism, urinary disorder, skin disease, diabetes, anemia, inflammation and allergic condition. It is used plant in folk and ayurvedic system of medicine. The main constituent of Guduchi is the Cordifolioside. structure of cordiofolioside is (shown in Figure 5)

Chemical Marker

Guduchi contains many classes of secondary metabolities like alkaloids, glycoside, diterpenoid lactones, steroid, sesquiterpenoids, aliphatic compounds (Maurya *et al.*, 1995) and immune-modulatory activities. The main component of the Guduchi is Cordifolioside A and syringin. (Wazir *et al.*, 1995)

Types of Chemical Marker

Active principle

Chemicals with well-defined properties and proven clinical utility

Example: Cordifolioside in *Tinospora Cordifolia*

Active marker

They are the constituent or community of constituents that contribute to efficacy and are considered to have pharmacological activity.

Example: Cordiofolioside in anti-inflammation activities

Analytical marker

They are a constituent or a group of constituents that help in the proper evaluation of raw materials that are used to achieve standardization and validation.

Analytical development

In this study, several analytical techniques have been reported for the analysis of cordiofolioside in Guduchi like HPLC. (Alam *et al.*, 2009)

Method validation

The procedure has been tested in accordance with ICH guidelines. Method validation is performed to ensure that the study is sufficient for its intended intent.

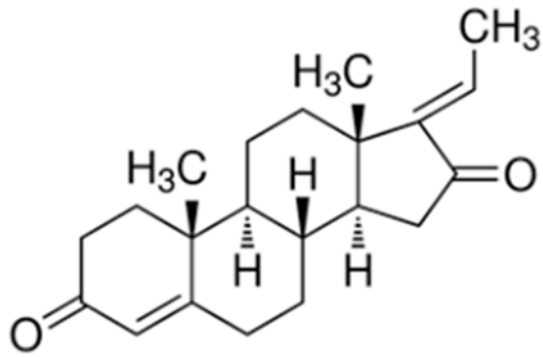

Figure 6: Guggulosterones Z

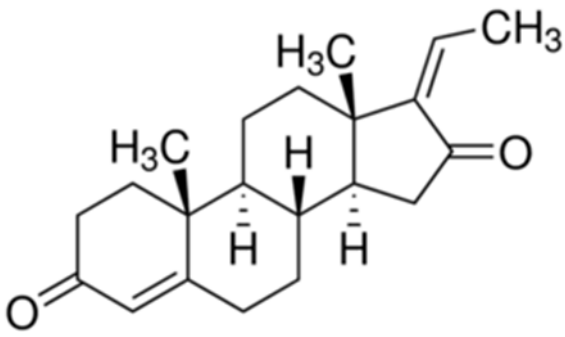

Figure 7: Guggulosterones E

A Shimadzu model HPLC was used, which included a quaternary LC-10A VP pump, variable wavelength programmable UV/VIS deductor SPD-10AVP column oven, SCL 10ABP device controller Rheodyne injector, and class-VP 5.032 software. The reverse step C18 zorax RP-HPLC column was used. (Reich and Schibli, 2007) The cost, sensitivity of the assay, and time needed for analysis all influence the solvent system's suitability. The method was validated

Table 8: Retention time, Correlation Co-efficient of HPLC for Tribulus Terrestris

Analytes	Retention time	Correlation coefficient (R2)
Diosgenin	12.6	0.999
Protodioscin	3.1	0.999

Table 9: Rf, Regression equation, R^2, LOD, LOQ data of Diosgenin, Protodioscin

Analyte	Rf	Regression equation	R^2	LOD	LOQ
Diosgenin	0.794	Y=9.636+205.69	0.9966	30.94	100
Protodioscin	0.44	Y=227.01+13.66X	0.996	40	100

Table 10: Precision of Cordiofolioside

Concentration	Intraday precision	Interday precision
5	0.30	0.32
10	1.04	1.06
20	1.24	1.30
40	0.22	0.26

Table 11: Linear regression data of Cordiofolioside

Linear Regression	Data
Linearity range	0.5-50
Regression equation	Y=29716X-4417.40
Correlation coefficient	0.997
Bias of Intercept	-0.0261

Table 12: LOD, LOQ of Cordiofolioside

Analyte	Retention time	LOD	LOQ
Cordifolio	9.52±1.03	0.18	0.55

Table 13: Rt, Linearity, Regression equation R^2, LOD, LOQ data of Guggulosterone

Guggulosterone	Rt	Linear range	Regression equation	R^2	LOD	LOQ
E-Guggulosterone	6.534	2.09	Y=5.3797X+0.534511	0.9988	1.284	3.890
G-Guggulosterone	8.531	2.13	Y=7.0974X+0.83915	0.9988	1.395	4.227

Table 14: Retention time, m/z of MS for Guggulsterone

S. No.	Analyte	Retention Time	m/z
1.	E-Guggulosterone	4.8	-313.2
2.	G-Guggulosterone	5.7	-313.8

by Accuracy, the precision of cordiofolioside (shown in Table 10), reproducibility, Linearity linear regression data of cordiofolioside (shown in Table 11), LOD, LOQ of cordiofolioside (shown in Table 12).

Guggulu

Guggulu is also known as *commiphora wightii.* Guggulu is having a variety of activities widely used in the treatment of osteoarthritis, hepatitis, dental infection, stomachic, anti-fertility activities, anti-rheumatic. Guggulu have been used since ancient times in the ayurvedic system of medicine. (Rege *et al.*, 1999) The active component in guggulu is oleo-gum-resin. The resin contains steroids Guggulosterols I, II, Guggulosterones Z and E (shown in Figure 6 and Figure 7).

Chemical Marker

Apart from small quantities of sesamin and other unspecified constituents, guggulu contains triterpenoids, steroids, diterpenoids, long-chain aliphatic tetrols, aliphatic esters, carbohydrates, ferulates, lignans and a number of inorganic ions. (Ahmed *et al.*, 2011)

Types of Chemical Marker

Active principle

Chemicals with well-defined properties and proven clinical utility

Example: Guggulsterone in Guggulu

Active marker

They are the constituent or community of constituents that contribute to efficacy and are considered to have pharmacological activity.

Example: E and Z Guggulsterone used in anti-inflammatory activity

Analytical marker

They are a constituent or a group of constituents that help in the positive identification of raw materials and are used to achieve standardization and validation.

Analytical development

In this study, several analytical techniques have been reported for the analysis of Guggulsterone in guggulu, like HPLC, LC-MS.

Method validation

The procedure has been tested in accordance with ICH guidelines. Method validation is performed to ensure that the study is sufficient for its intended intent. (EMEA, 1995)

The gemini C18 column was used for HPLC research. Degassing and preparing the mobile process To acquire the chromatogram for identification of Guggulsterol I, II, E, and Z Guggulsterone, the PDA deductor was set by optimising wavelength to provide the best answer for all samples. Using Shimadzu's Lab solution programme 5.53 SP 3C, the chromatogram was acquired and compared. Rt, Linearity, Regression equation R^2, LOD, LOQ data of Guggulosterone (shown in Table 13).

Guggulsterol I, II, E, and Z guggulsterone were separated and identified chromatographically using a gemini C18 column with a photo diode array deductor and volume deductor (MS) - Retention time, m/z of MS for Guggulsterone (shown in Table 14). In both positive and negative ionisation, the MS was set to ion source electrical spray ionisation. To obtain the chromatogram, the experiment was carried out by optimising wavelengths to give the best answer for all samples.

CONCLUSION

The markers are essential in the Ayurvedic formulation of anti-inflammatory drugs. The chromatographic approach is used to estimate the commonly used markers Sunthi, Guduchi, Serrata, Guggulu, and others. Chromatographic analysis based on markers The issue of comparing integrated sameness/ difference of controlling the stability of available drugs can only be addressed by spectroscopic examination. For the consistency of herbal medicine, studies on the relationship between quantification and efficacy of herbal medicine have been conducted. For many makers, the hyphenated technique is not still developed. Increasing Analytical techniques make many researches prone to be more successful in future studies promotes Quality, safety, efficacy.

Conflict of Interest

The authors declare that they have no conflict of interest.

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REFERENCES

Ahmed, R., Ali, Z., Wu, Y., Kulkarni, S., Avery, M., Choudhary, M., Khan, I. 2011. Chemical Characterization of a CommercialCommiphora wightiiResin Sample and Chemical Profiling to Assess for Authenticity. *Planta Medica*, 77(9):945–950.

Alam, P., Ali, M., Singh, R., Madhurima, Ahmad, S., Shakeel, F. 2009. A Validated HPLC Method for Estimation of Cordifolioside A in Tinospora cordifolia, Miers and Marketed Formulations. *Journal of*

Chromatographic Science, 47(10):910–913.

Alam, P., Alqasoumi, S. I., Abdel-Kader, M. S. 2016. Simultaneous Determination of Menthol and Eucalyptol by the Densitometric HPTLC Method in Some External Analgesic Formulations. *Journal of Chromatographic Science*, 54(1):58–63.

Ammon, H. P. T. 2016. Boswellic acid and their role in chronic inflammatory diseases. *Adv.Exp.Med.Biol*, 928:291–327.

EMEA 1995. ICH Topic Q2 R1 validation of Analytical Procedures: Text and Methodology. *European Medicine Agency*, pages 1–15.

Engels, G. 2010. Frankincense, herbal gram. *J. Am. Bot. Coun*, 88:1–4.

Govintharajan, V. S. 1982. Ginger-chemistry, technology and quality evaluation: part I. *Crit rev sci nutr*, 17(1):1–96.

Gupta, P., Nagore, D., Kuber, V., Purohit 2012. A validated RP-HPLC method for the estimation of diosgenin from a polyherbal formulation containing Tribulus terrestris linn. *Asian Journal of pharmaceutical and clinical Research*, 5(4):91–94.

Hostettmann, K. 2014. Handbook of chemical and Biological Plant Analytical Methods. In and others, editor, *Drug Discovery and Development*, page 1176. Wiley. Wiley, First Edition. ISBN: 978-1-118-87498-1.

Maurya, R., Wazir, V., Tyagi, A., Kapil, R. S. 1995. Clerodane diterpenoids from Tinospora cordifolia. *Phytochemistry*, 38(3):659–661.

Mustafa, T., Srivastava, K. C. 1990. Possible leads for arachidonic acid metabolism altering drugs from natural products. *J drug Dev*, 3:47–60.

Rege, N. N., Thatte, U. M., Dahanukar, S. A. 1999. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine. *Phytotherapy Research*, 13(4):275–291.

Reich, E., Schibli, A. 2007. High-performance thin-layer Chromatography for Analysis of Medical Plants. First Edition. *Thieme Medical Publication Inc.* . ISBN: 1588904091.

Schzetner, H. A., Rios, D. 2007. High-performance liquid chromatographic analysis of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in ginger-containing dietary supplements, spices, teas, and beverages. *J Chromatogr B Analyt Technol Biomed Life Sci*, 856(1-2):41–47.

Wazir, V., Maurya, R., Kapil, R. S. 1995. Cordioside, a clerodane furano diterpene glucoside from Tinospora cordifolia. *Phytochemistry*, 38(2):447–449.