Determination and Quantification of Ferulic acid in *Ferula asafoetida* by a Validated HPTLC Method

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ABSTRACT

Phenolic acids are a group of compounds categorized as plant secondary metabolites widely distributed in plant kingdom and which furnishes with a number of applications. The oleo gum resin obtained from *Ferula asafoetida* commonly known as 'HING' possess ferulic acid which is one of a phenolic acid as an ingredient with a potent pharmacological activity. In the present study an attempt has been made to develop an HPTLC method for the quantitative estimation of ferulic acid from *Ferula asafoetida*. The method employed TLC aluminum plates precoated with silica gel $60F_{254}$ as stationary phase. Linear ascending development with toluene-ethyl acetate-formic acid in the ratio 60:40:10 as mobile phases were performed at room temperature (25 ± 2)°C in a twin trough chamber saturated with mobile phase vapour. The system was found to give a compact spot for ferulic acid (Rf value of 0.48 ± 0.02). The detection was carried out at a wavelength of 319 nm. Linear regression analysis of calibration plot showed good co-relation between peak area and concentration with a co-relation coefficient r^2 = 0.989 in the range of 200 ng/spot – 700 ng/spot. The developed method was validated for various validation parameters such as linearity, precision, accuracy as recovery, specificity, detection and quantification limits. The developed HPTLC method can be employed as a quality control tool for checking the purity of asafoetida resin powder, commercially available asafoetida and formulations comprising of asafoetida.

Keywords: HPTLC, *Ferula asafoetida*, ferulic acid, method validation.

INTRODUCTION

Ferula asafoetida syn *Ferula foetida regel* the oleo gum resin of which is commonly known as 'HING' belonging to family Umbelliferae is perennial plant which grows to about 2 m by 1.5 m and requires dry or moist soil. The dried latex (oleo-gum-resin) obtained by making deep incisions in the roots and rhizomes are preferred for medicinal as well as for culinary purpose.[1] Asafoetida chiefly comprises chiefly of resin (40 to 65%), gum (20 to 25%) and volatile oil (4 to 20%). The resin portion consists of assaresinotannol in free or in combined form with ferulic acid and volatile polysulfides. It also contains free ferulic acid and galbanic acid as well.[2] Literature survey also revealed the various therapeutic activity of asafoetida such as the antispasmodic,[3,4] antifungal,[5] antioxidant,[6,10] anti-diabetic,[7] anti-microbial,[8] antiulcer,[9] antihaemolytic,[10] chemopreventive,[11,12] antiviral.[13] The drug has been employed as an expectorant, as remedies for headache, for treatment of asthma and is also used as an antidote for snake bite when mixed with garlic.[14] Ferulic acid is one of the active constituent of *Ferula asafoetida* which is concerned with the anticancer and antioxidant activity of asafoetida.[15,16] Literature studies revealed very few methods for quantification of ferulic acid from asafoetida. The current practice of the standardization of herbal drugs and preparations doesn't include any standard identification tests or any analytical procedures to maintain the quality and purity of the same. The pharmacopoeial standards in ayurvedic pharmacopoeia are not enough to assure the quality of herbal drugs and preparation. Hence an attempt has been made to develop a simple, sensitive, and cost

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effective validated HPTLC method for quantification of ferulic acid from *Ferula asafoetida*. HPTLC is a valuable tool for investigation of herbal products in comparison to the different aspects of quality. The major advantage that HPTLC offers is that a large number of samples can be analyzed by using comparatively less amount of mobile phase.[17]

MATERIALS AND METHODS

Chemicals

AR grade chemicals such as methanol, toluene, ethyl acetate, formic acid were obtained from S.D. Fine chemicals, Mumbai. Ferulic acid of 98% purity was purchased from P.C. Chem (Mumbai, India).

Plant material

The dried oleo-gum-resin of asafoetida was obtained from Yucca Enterprises Mumbai. The oleo-gum-resin was powdered and kept in an air tight container.

Conditions of chromatography

HPTLC was carried out on 10 cm × 10 cm TLC aluminum plates coated with 200-μm layer thickness of silica gel $60F_{254}$ (E. Merck, Germany). Samples were applied as 6 mm width bands using Camag 100 microlitre sample syringe (Hamilton, Switzerland) with a Camag Linomat 5 applicator (Camag, Switzerland). The samples were applied with a constant application rate of 150 nl/s. Linear ascending development with toluene: ethyl acetate: formic acid as mobile phases in the ratio of 60:40:10 was carried out in a twin trough glass chamber (10 × 10 cm) pre-saturated with mobile phase vapours for 10 mins at room temperature (25 ± 2ºC). The plates were developed to a distance of 80 mm. After development the plates were air dried. The developed dried plates were scanned using Camag TLC scanner 3 at 319 nm in the absorbance mode and operated by winCATS software (version 1.4.1). The source of radiation was deuterium lamp. The slit dimensions were 5 mm × 0.45 mm and the scanning speed was 100 mm/s.

Preparation of standard ferulic acid solution

A stock solution of ferulic acid (1 mg/ml) was prepared by dissolving 10 mg of accurately weighed ferulic acid in methanol and making up the volume to 10 ml with methanol in a 10 ml volumentric flask. It was further subjected for dilution with methanol in order to get a solution of 100 μg/ml.

Calibration curve for ferulic acid

Different volumes of the diluted solution (2, 3, 4, 5, 6 and 7 μl) were applied in duplicate in order to get 200 ng/spot – 700 ng/spot of ferulic acid. The plate was developed and scanned as per the aforesaid chromatographic conditions. The peak areas were recorded. The calibration curve was plotted by peak area data and their corresponding concentration by linear least square regression analysis.

Preparation of methanolic extract of *Ferula asafoetida*

Accurately weighed 25 g of asafoetida powder was extracted with 100 ml methanol and it was subjected for filtration. The filtrate was evaporated to get a brown extract, which was used for further analysis.

Preparation of sample solution

25 mg of the methanolic extract was accurately weighed and transferred to a 25 ml volumentric flask containing about 10 ml of methanol. The mixture was sonicated for 10 min and the required volume was made upto 25 ml by methanol. The resulting sample solution was filtered through a 0.45 μ filter. 10 μl of filtered solution was then applied as triplicate on the TLC plate and then subjected for development and scanning.

Method validation

The developed method was validated as per ICH guidelines (Q2R1).[18] The various parameters that were checked are as follows:

Precision

The precision of system was determined by measuring the repeatability of sample application and measurement of peak areas for six replicates of the same band (600 ng/spot). To evaluate the intraday precision, six samples at three different concentrations (500, 600 and 700 ng/spot) were analyzed on the same day. The interday precision was evaluated by analyzing the samples on three different days. The results of the precision studies were reported as relative standard deviation (% RSD) of peak area.

Limits of detection and quantification

The limit of detection (LOD) of an analytical procedure is the lowest amount of analyte in a sample which can be detected but cannot be quantitated as an exact value as such. LOD was calculated using the formula 3.3 × (standard deviation of y-intercept)/slope of the calibration curve. The limit of quantification (LOQ) of an analytical procedure can be defined as the lowest amount of an analyte in a sample which can be quantitatively determined. LOQ can be calculated by using the formula 10 × (standard deviation of y-intercept)/slope of calibration curve.

Robustness

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in the method parameters. It was studied in triplicate at 500 ng/spot and 600 ng/spot by making small changes in mobile phase composition and the mobile phase saturation time. The final results were examined by calculation of RSD (%) of peak areas. Mobile phases prepared from toluene, ethyl acetate and formic acid in different proportions (58:42:10) and (62:38:10) were used for development. Mobile phase saturation time were investigated at 10 ± 2 min (8, 10, 12) respectively.

Accuracy as recovery

The accuracy was determined by spiking the preanalysed samples with extra 80%, 100% and 120% of standard ferulic acid. Three determinations were performed at each level in order to check the recovery of ferulic acid at different levels.

Specificity

The specificity of the method was determined by analyzing and comparing the Rf and the spectra of the ferulic acid band from a sample with that of the standard.

RESULTS AND DISCUSSION

Development of a suitable mobile phase

The TLC procedure was employed for selection of a suitable mobile phase for quantification of ferulic acid in asafoetida. The best suitable mobile phase was optimized with varying ratios of toluene, ethyl acetate and formic acid.[19] The best possible composition which gave good resolution, dense, compact, sharp and well separated spots of ferulic acid as well as well defined peaks at an Rf of 0.48. Chromatographic conditions which gave reproducible Rf values and better resolution was selected for chromatographic purpose (Figures 1, 2, 3).

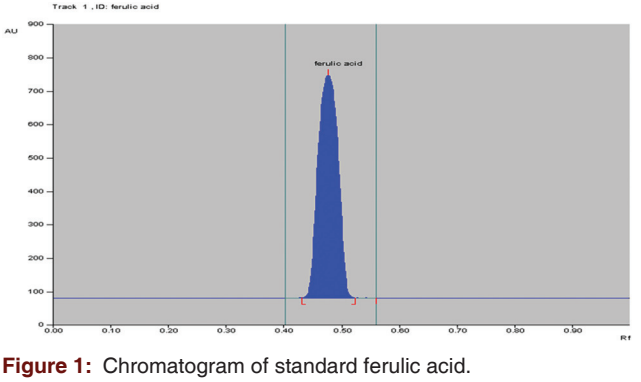

Figure 1: Chromatogram of standard ferulic acid.

Validation of method linearity

A linear relationship was obtained when a graph was plotted for concentration v/s peak area with a co-relation coefficient value r^2 = 0.989 in the range of 200 ng/spot – 700 ng/spot (Table 1) (Figure 4).

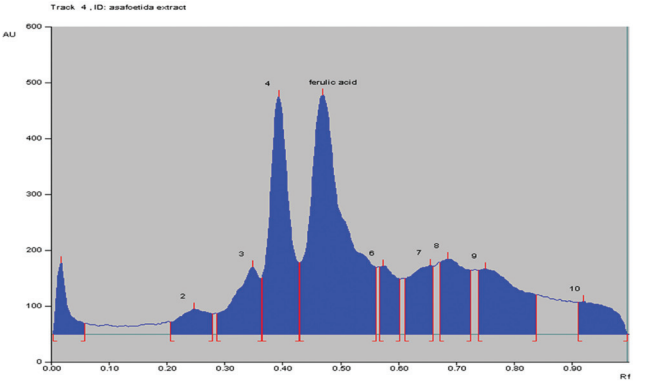

Figure 2: Chromatogram of methanolic extract.

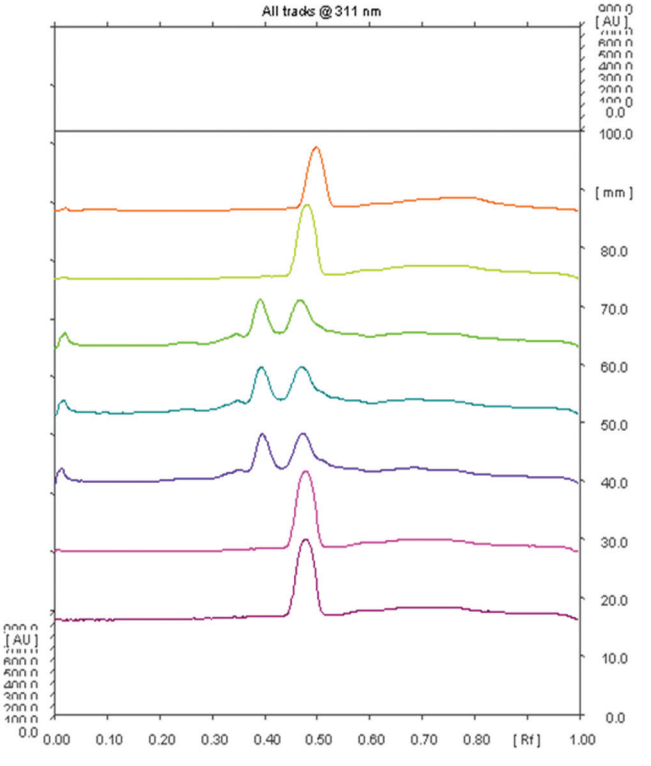

Figure 3: Overlaying spectra of ferulic acid standard and from the extract.

Table 1: Regression Analysis (n = 2)	
Linearity Range	200 ng/spot – 700 ng/spot
Regression equation	y = 18.26x + 5949
Co-relation coefficient r^2	0.989
Slope	18.26
Intercept	5949

Precision

The % RSD for repeatability of sample application (600 ng/spot) and measurement of peak areas were 0.98% and 0.865% respectively. The % RSD inter day and intraday variation was found to be less than 2% which indicated good precision for the developed method (Tables 2, 3).

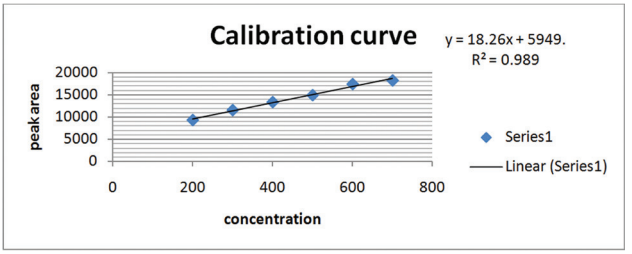

Figure 4: Calibration curve of ferulic acid.

Table 2: Method Validation	
Instrument precision	% RSD = 0.865
Method precision	% RSD = 0.98
LOD	56 ng/spot
LOQ	164 ng/spot

Limit of detection and quantification

The limit of detection (LOD) was found to be 56 ng/spot and limit of quantification was found to be 164 ng/spot respectively indicating adequate sensitivity of the method (Table 3).

Robustness

The % RSD of the peak area were calculated in triplicate for changes in mobile phase composition and the duration of saturation for 500 ng/spot and 600 ng/spot. The values of % RSD were less than 2% which were obtained after making some deliberate changes in the developed method which indicated that the developed method is robust (Table 4).

Accuracy as recovery

The proposed method when used for quantification of ferulic acid from methanolic extract after spiking with standard afforded average recovery of 101.8% (Table 5).

Specificity

There were no other interfering spots by other constituents of the extract at the Rf values of the standard ferulic acid at Rf = 0.48. The spectrum of standard ferulic acid and the corresponding spot present in sample matched

Table 3: Intraday and Inter Day Precision

Amount ng/spot	Intraday precision		Interday precision	
	Mean area	% RSD	Mean area	% RSD
500	14628.12	0.755	14216.23	0.85
600	16508.12	0.53	16536.5	0.96
700	17627.35	0.64	17363.8	0.64

Table 4: Robustness of the Method

Parameters	% RSD	
	500 ng/spot	600 ng/spot
Mobile phase composition		
Toluene: Ethyl Acetate: Formic acid (5.8:4.2:1)	0.404	0.37
Toluene: Ethyl Acetate: Formic acid (6:4:1)	0.707	0.78
Toluene: Ethyl Acetate: Formic acid (6.2:3.8:1)	0.94	0.704
Duration of saturation time (min)		
8	0.311	0.298
10	0.57	0.63
12	0.207	0.63

Table 5: Accuracy as Recovery

Amount of marker present (ng) A	Amount of marker added (ng) B	Total amount of marker (ng) (A + B)	Amount of marker found (ng)	Recovery %	Average recovery %
330	260	590	600.86	101.8	
330	330	660	679.33	102.9	101.8
330	390	720	725.66	100.8	

exactly and was found to be overlapping, indicating no interference from other constituents (Figures 5, 6, 7).

Estimation of ferulic acid in extract

The chromatogram of the standard ferulic acid showed a single spot at Rf value of 0.48. The chromatogram of the extract showed no interferences from other constituents of the respective extract. The amount of ferulic acid in the asafoetida was calculated by making use of the calibration curve and was found to be 0.117% (Figure 3).

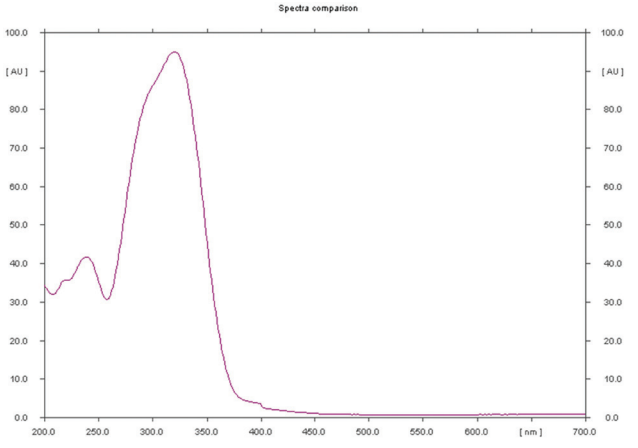

Figure 5: Spectra of standard ferulic acid at Rf 0.48.

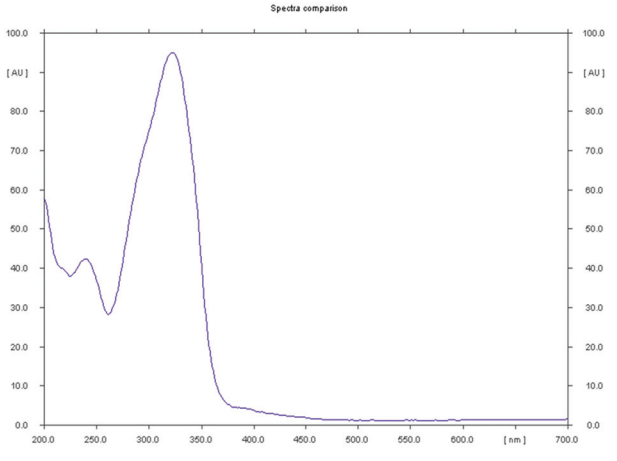

Figure 6: Spectra of ferulic acid from methanolic extract at Rf 0.48.

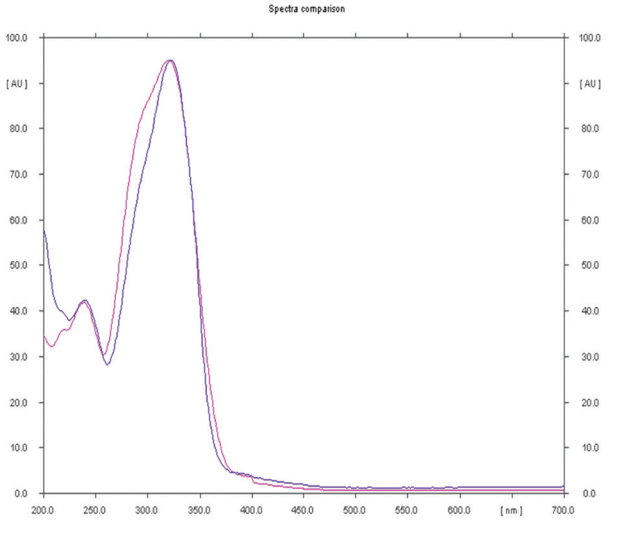

Figure 7: Overlay of standard ferulic acid and ferulic acid from methanolic extract at Rf 0.48.

CONCLUSION

The proposed HPTLC method was found to be rapid, simple, precise, specific and reliable for the determination of ferulic acid. The mobile phase employed for method development effectively resolves ferulic acid hence the developed method can be utilized for analysis of ferulic acid in herbal extracts. Statistical analysis proves that the developed method is reproducible. The method is economical as it use very small amount of mobile phases and the sample clean up procedure associated with it is minimal. The developed HPTLC method can be widely used for quality control of *Ferula assafoetida* and formulations comprising of the same.

REFERENCES

1. Shah and Seth. Textbook of Pharmacognosy and Phytochemistry. 1st ed. New York: Elsevier; 2010.
2. Wallis TE. Textbook of Pharmacognosy. 5th ed. New York: CBS publisher; 2004.
3. Mohammad F, Freshteh F, Hassanabad ZF. Antispasmodic and hypotensive activity of *Ferula asafoetida* gum extract. J Ethnopharmacol 2004; 91:321–4.
4. Gholamnezhad Z, Byrami G, Boskabady MH. Possible mechanism of the relaxant effect of asafoetida. Avi J Phyto Med 2012; 2:10–16.
5. Houghton PJ, Apendino G, Maxia L. A meroterpenoid NF-κβ inhibitor and drimane sesquiterpenoids from asafoetida. J Nat Pro 2006; 69:1101–4.
6. Dehpour AA, Ebrahimzadeh MA, Fazel NS. Antioxidant activity of methanolic extract of *Ferula assafoetida* and its essential oil composition. Gra Y Ace 2009; 60:405–12.
7. Abu Zaiton AS. Anti-diabetic activity of *Ferula assafoetida* extract in normal and alloxan-induced diabetic rats. Pak J Bio Sci 2010; 13:97–100.
8. Mishra N, Behal KK. Antimicrobial activity of some spices against selected microbes. Int J Pharm Pharm Sci 2010; 2:187–96.
9. Alqasoumi S, Al-Dosari M, Al-Howiriny T. Gastric antiulcer activity of a pungent spice *Ferula assafoetida* in rats. FARMACIA 2011; 59:750–9.
10. Nabavi SM, Ebrahimzadeh MA, Nabavi SE. Antioxidant and antihaemolytic activity of *Ferula foetida regel*. Eur Rev Med Pharmacol Sci 2011; 15:157–64.
11. Mohammad S, Alam A, Sultana S. Asafoetida inhibits early events of carcinogenesis: A chemopreventive study. Life Sci 2001; 68:1913–21.
12. Mishra N, Behal KK. Chemopreventive activity of some spices against selected cell line. Der Pharm Sin 2011; 2;31–5.
13. Lee CL, Chiang CL, Cheng HL. Influenza A (H_1N_1) and cytotoxic agents from *Ferula assafoetida*. J Nat Prod 2009; 30:1–5.
14. Bhattacharjee SK. Medicinal Herbs and Flowers. 1st ed. India: Avishkar publication. 2005. Pg no. 142.
15. Baskaran N, Manoharan S, Balakrishnan S. Chemopreventive potential of ferulic acid in 7,12-dimethylbenz[a]anthracene-induced mammary carcinogenesisin Sprague-Dawley rats. Eur J Pharmacol 2010; 637:22–9.
16. Cheng CY, Su SY, Tang NY. Ferulic acid provides nueroprotection against oxidative stress-related apoptosis after cerebral ischemia/perfusion injury by inhibiting ICAM-1 mRNA expression in rats. Brain Research 2008; 1209:136–50.
17. Reidh E and Schibli A. High performance thin layer chromatography for the analysis of medicinal plants. New York: Thieme publishers; 2007. 130,162.
18. ICH. Q2 (R1). Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization, Geneva, Nov 2005.
19. Khatoon S, Singh H, Singh K. TLC evaluation and quantification of phenolic compounds in different parts of *Dendrophthoe falcate Etting*. J Plan Chrom 2010; 23:104–7.