

# Development of HPTLC Method for Estimation of Gallic Acid and Bergenin in *Actaea acuminata* Roots

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## ABSTRACT

**Introduction:** The major drawback associated with herbal drugs is to get their reproducible therapeutic efficacy. Standardization of herbal drugs with marker compounds seems to be a viable solution to achieve their consistent therapeutic effects. **Objectives:** Standardization of roots of *Actaea acuminata*, a traditional medicinally promising plant, using selected marker compounds. **Methods:** *A. acuminata* was standardized by estimating the content of gallic acid and bergenin in its roots using HPTLC system. Soxhlet extraction process was used to prepare methanol extract of plant material. **Results:** Gallic acid and bergenin, in methanol extract, were well resolved on TLC plates using chloroform: methanol (7:3; scanned at 292 nm) and chloroform: methanol (17:3; scanned at 280 nm), respectively, as solvent systems. The content of marker compounds was quantitatively determined by TLC densitometric methods, which were validated based on the parameters described in ICH guidelines. Gallic acid and bergenin were estimated to be 0.1242% and 0.8010% w/w, respectively, in *A. acuminata* roots. **Conclusion:** Standardized *A. acuminata* roots can be utilized for the development of clinical medicine.

**Key words:** *Actaea acuminata*, Bergenin, Gallic acid, HPTLC, Standardization.

## INTRODUCTION

The demand for the herbal drugs has increased enormously in last few decades. This increasing demand has created pressure on herbal drug industries to set up analytical research and development laboratories to standardize herbal products on the basis of marker compounds to meet the stringent international standards. The standardization of herbal products is carried out by estimating the content of marker compounds using advanced and sophisticated instruments such as HPLC, HPTLC or LC-MS.<sup>1</sup>

*Actaea acuminata* H. Hara (Ranunculaceae) has long been used as a therapy for mental disorders, inflammation, ovarian neuralgia, rheumatism, chorea, uterine tenderness, asthma, rheumatic fever, skin diseases, cough, lumbago, sciatica, scrofula, constipation and vomiting.<sup>2-5</sup> The exhaustive survey of literature reveals that marker

based standardization of *A. acuminata* has never been carried out. Therefore, the present investigations were designed to standardize plant material on the basis of marker compounds.

## MATERIALS AND METHODS

### Plant Material

The identity of plant material, procured from K. R. Indo German American Trading Company, Kurukshetra, India in the month of August, 2015, was confirmed as *Actaea acuminata* through Botany Department of Punjabi University Patiala, India (SPL-110/Bot, dated 15-09-2015). A specimen copy (No. 102) of HPTLC fingerprint profile of plant's methanol extract was deposited to Museum-cum-Herbarium of Department of

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### Solvents, Chemicals, Reagents and Instruments

Various solvents, chemicals and reagents, of AR grade (E Merck, New Delhi, India), were used in present analytical studies. HPTLC system comprising Camag Linomat 5 applicator, Camag dipping chamber and Camag TLC Scanner 4 with Wincats software version 1.4.8.2031 (Anchrom Enterprises India Pvt. Ltd.) and digital weighing balance (KERN, 120-4N, Germany) were used for analytical studies. A pack of 25 pre-coated plates (0.2 mm thickness, aluminum base, 20 × 20 cm) of E Merck was purchased. The plates were cut into different sizes using sharp blade cutter as per requirement in HPTLC studies.

### HPTLC Method Development

#### Markers Solutions

The stock solution of gallic acid and bergenin was individually prepared by dissolving accurately weighed 5 mg of each compound in 5 ml of methanol.

#### Test solution

Exhaustive extraction of coarsely powdered roots of *A. acuminata* (10 g) was carried out in Soxhlet apparatus using methanol solvent. After filtration, the methanol extract was concentrated under reduced pressure to 10 ml. The concentrated methanol extract was then transferred to a volumetric flask (250 ml) and the final volume was adjusted to the mark of flask with methanol.

#### Standard Plot

Six dilutions of different concentrations for gallic acid (10, 14, 18, 22, 26 and 30 µg/ml) and bergenin (10, 15, 20, 25, 30 and 35 µg/ml) were produced by diluting their stock solutions with methanol. Each dilution (10 µl) of gallic acid and bergenin was loaded on pre-coated TLC plates in the form of band (1 cm). TLC plates were developed for gallic acid using solvent system – chloroform: methanol (7:3) and for bergenin using solvent system – chloroform: methanol (17:3). The developed TLC plate for gallic acid and bergenin was scanned at 292 and 280 nm, respectively and corresponding peak areas were recorded.

#### Estimation of Marker Compounds

Ten µl of test solution was loaded, in triplicate, in the form of band (1 cm) on TLC plate. Similar procedure as explained in the section “Preparation of standard plot” was adopted for development and scanning of TLC plate and recording of peak areas of gallic acid and bergenin in the test sample. The regression equations

obtained from standard plots of gallic acid and bergenin were used for calculating their percentage content in test sample.<sup>6</sup>

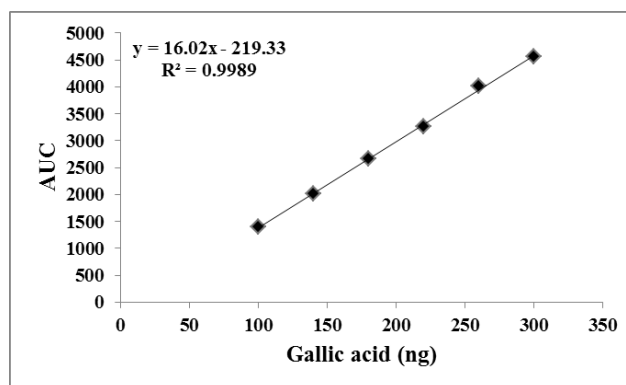
### TLC Densitometric Method Validation Studies

The developed methods were validated for the parameters described in ICH guidelines.<sup>7</sup>

## RESULTS AND DISCUSSION

The increased demand of herbal products in last two decades has forced Indian herbal industries to produce scientifically standardized herbal drugs to meet global standards. In order to assess the quality and safety of herbal drugs, their standardization is essential.<sup>8</sup> Establishment of chemo profile of herbal drugs and their products using analytical techniques such as HPTLC or HPLC is required not only for the detection of active ingredients but also for standardization of such materials.<sup>9,10</sup> HPTLC has several advantages over other techniques such as crude samples containing multi-components can be easily analysed by HPTLC method; colored compounds can be easily separated by this technique; high output, time saving and a rapid low cost analysis; wide range of solvents can be used as mobile phase; easy to perform two-dimensional separations; specific colour sensitive reagents can be used for the detection of separated spots; and method development and validation is easy as it involves some basic steps of thin layer chromatography.

HPTLC methods were developed for estimation of gallic acid and bergenin in *A. acuminata* roots and validated as per ICH guidelines. Standard plots were prepared between different concentrations of gallic acid and bergenin versus their peak areas after scanning at 292 and 280 nm respectively (Figures 1-2). Linearity of standard plots of gallic acid and bergenin was achieved between 100-300 ng and 100-350 ng,



**Figure 1: Standard plot between amount of gallic acid versus area under the curve (AUC).**

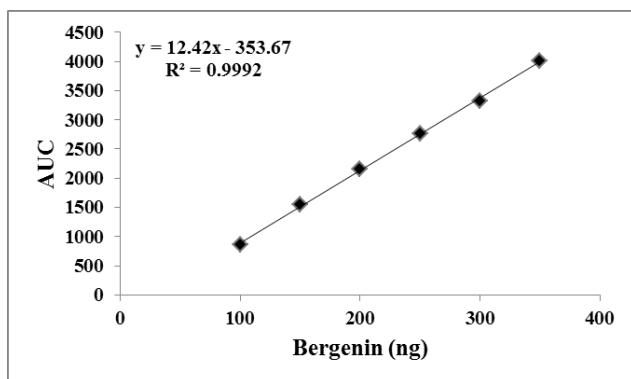


Figure 2: Standard plot between amount of bergenin versus area under the curve (AUC).



Figure 3: TLC fingerprint profile of gallic acid and methanol extract.

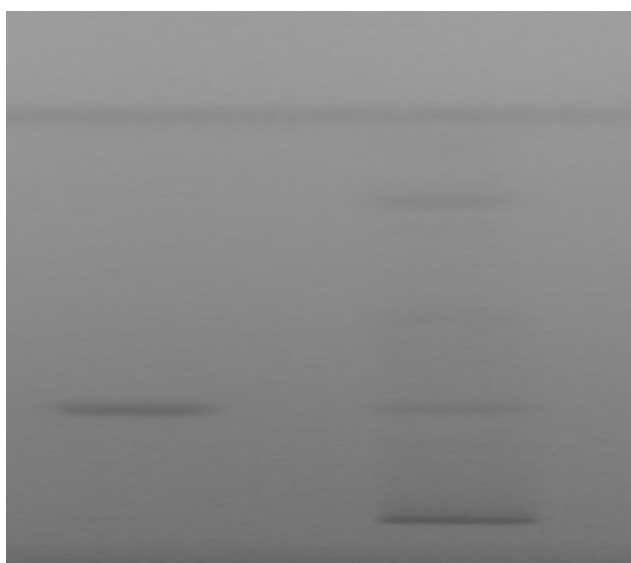


Figure 4: TLC fingerprint profile of bergenin and methanol extract.

Table 1: Percentage content of marker compounds in *A. acuminata* roots.

Marker compound	Mean <sup>n</sup> percentage content of marker $\pm$ S.D.
Bergenin	0.8010 $\pm$ 0.00001
Gallic acid	0.1242 $\pm$ 0.00000

*n* = 3

Table 2: Method validation parameters of TLC densitometric method.

Parameter	Bergenin	Gallic acid
Instrumental precision (% CV, <i>n</i> =7)	0.50	0.49
Repeatability (% CV, <i>n</i> =5)	1.08	0.42
Coefficient of determination	0.9992	0.9989
Linearity (ng)	100-350	100-300
LOD (ng)	21	9
LOQ (ng)	62	26
Intra-day precision (% CV, <i>n</i> =9)	0.98	0.81
Inter-day precision (% CV, <i>n</i> =9)	1.11	1.23
Accuracy (average % recovery)	98.60	98.93
Specificity	Specific	Specific

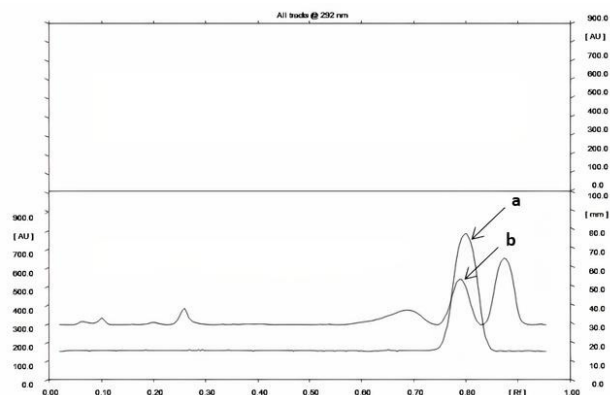


Figure 5: TL chromatogram of gallic acid (a) and crude methanol extract of *A. acuminata* roots (b).

respectively. Various solvent systems were tried, but the best resolution of gallic acid and bergenin in methanol extract was observed in chloroform: methanol (7:3) and chloroform: methanol (17:3) respectively. TLC fingerprint profiles of gallic acid and bergenin along with methanol extract are shown in Figure 3 and Figure 4 respectively. The marker compounds were estimated in triplicate and percentage content (w/w; expressed as mean  $\pm$  S.D.) of gallic acid and bergenin is presented in Table 1. The validation parameters for developed

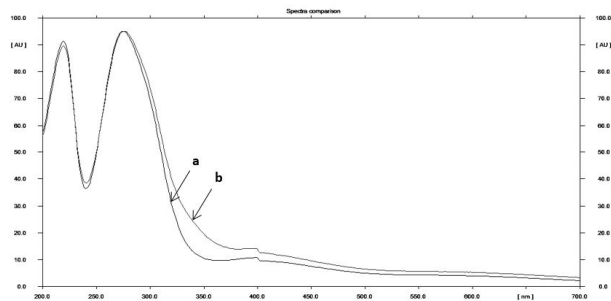


Figure 6: UV spectra overlay of gallic acid (a) and crude methanol extract of *A. acuminata* roots (b).

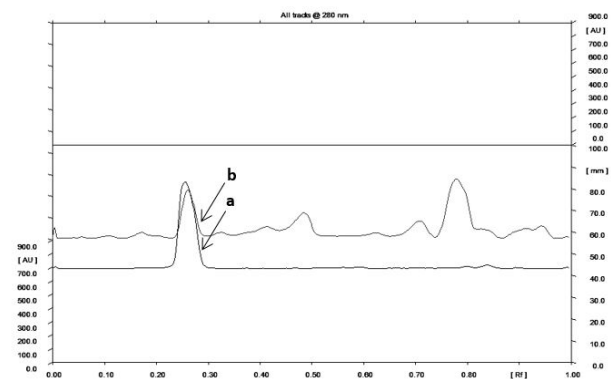


Figure 7: TL chromatogram of bergenin (a) and crude methanol extract of *A. acuminata* roots (b).

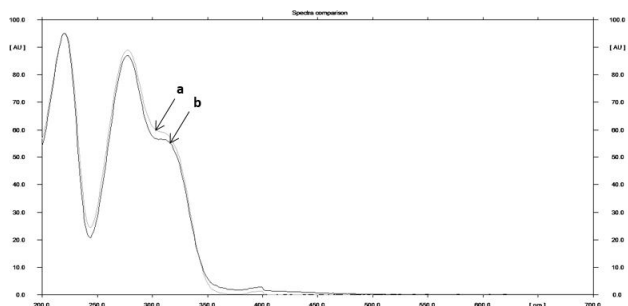


Figure 8: UV spectra overlay of bergenin (a) and crude methanol extract of *A. acuminata* roots (b).

HPTLC methods for estimation of gallic acid and bergenin are shown in Table 2. No interference was observed in ultraviolet (UV) spectra and thin layer (TL) chromatogram overlays of marker compounds and methanol extract (Figures 5-8). Average percent recovery was found to be more than 98% w/w for both marker compounds (Table 3). These observations inferred that analytical HPTLC methods are specific and accurate for each standard marker. Finally, it can be suggested that all validation parameters complied with the limits prescribed in ICH guidelines.

### CONCLUSION

Gallic acid and bergenin, bioactive compounds of *A. acuminata*, have been used as marker compounds for standardization purpose. The use of such standardized *A. acuminata* roots in herbal formulations can be utilized for the development of clinical medicine.

### ACKNOWLEDGEMENT

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### CONFLICT OF INTEREST

The authors have no conflict of interests.

### ABBREVIATIONS

**μl:** Microliter; **cm:** Centimeter; **HPLC:** High Pressure Liquid Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **LC-MS:** Liquid Chromatography–Mass Spectroscopy; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **nm:** Nanometer; **SD:** Standard Deviation; **TLC:** Thin Layer Chromatography.

Table 3: Recovery studies of marker compounds.

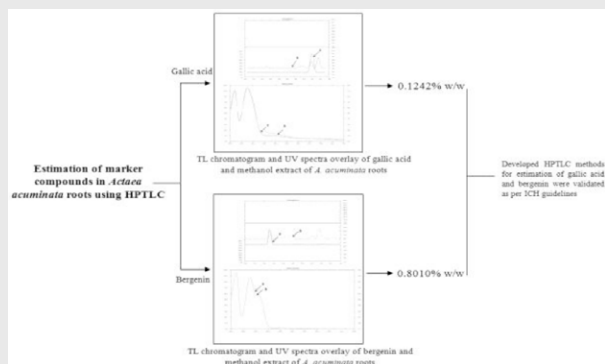
Compound	Initial amount			Amount detected (mg) (Mean <sup>n</sup> ± S.D.)	Percent recovery	Average percent recovery (Mean <sup>n</sup> ± S.D.)
	In percent	Amount of compound (mg) [equivalent to 10 g plant material taken]	Spiked amount (mg)			
Bergenin	0.8010	80.10	64.08 (80 %)	142.85 ± 0.02	99.07	98.60 ± 0.50
			80.10 (100 %)	157.11 ± 0.08	98.07	
			96.12 (120 %)	173.89 ± 0.07	98.67	
Gallic acid	0.1242	12.42	9.93 (80 %)	22.10 ± 0.21	98.88	98.93 ± 0.32
			12.42 (100 %)	24.50 ± 0.08	98.63	
			14.90 (120 %)	27.12 ± 0.04	99.26	

n = 3

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## PICTORIAL ABSTRACT



## SUMMARY

An attempt was made to develop analytical methods on HPTLC system for estimation of the contents of marker compounds, i.e., gallic acid and bergenin in *Actaea acuminata* roots. Gallic acid and bergenin were estimated to be 0.1242% and 0.8010% w/w, respectively, in *A. acuminata* roots.

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