# QUANTIFICATION AND VALIDATION OF GALLIC ACID CONTENT IN TRI-PHALA EXTRACT AND EFFERVESCENT TABLET BY HPTLC

Benjaporn Chainethvanich[1], Worawan Saingam[2], Prasan Tanguenyongwatana[1]

[1]*Faculty of Oriental Medicine, Rangsit University, Pathumthani 12000, Thailand*
[2]*Faculty of Pharmacy, Rangsit University, Pathumthani 12000, Thailand*

**KEYWORDS:** Gallic acid, Tri-phala, Effervescent tablet, HPTLC, method validation



## INTRODUCTION

Gallic acid is a major compound in many herbs that have been used in Traditional Thai Medicine. This compound is known to have anti-oxidation, anti-inflammatory, antimutagenic and anticancer activities[1, 2]. In Thai traditional formula, Tri-phala has been used to adjust the balance of the elements in the body, detoxification and rejuvenation[3]. Tri-phala composes of three herbs which are *Terminalia bellerica* Roxb., *Terminalia chebula* Retz, and *Phyllanthus emblica* L. All of them have gallic acid as an active constituent [4-6]. This research developed a high performance thin-layer chromatography (HPTLC) method for the simultaneous quantification of gallic acid in Tri-phala extract and effervescent tablet. The tablet weights size was 4000 mg and composed of 25% of Triphala extract. The method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ).

## MATERIALS AND METHODS

***Instruments and reagents*** Reagents and solvents were reagent grade and used without further purification. Gallic acid was purchased from Aldrich (USA). Freeze Dryer was performed on EYELA FD-1(Tokyo Rikakikai, Japan). HPTLC was performed on pre-coated HPTLC silica gel $GF_{254}$ plates cat. No.1.05548.0001 (Merck, Germany). Spotting device was Linomat 5 automatic sample spotter (CAMAG, Muttenz, Switzerland). TLC chamber was glass twin-trough chamber (20 × 10 cm.) (CAMAG, Switzerland). Densitometer was TLC scanner 3 with winCATS software (CAMAG, Switzerland). Syringe was 100 µL size (Hamilton, Bonaduz, Switzerland).

***Plant materials*** The plant materials of *Terminalia bellerica, Terminalia chebula* and *Phyllanthus emblica* were bought from local drugstore in Nonthaburi province, Thailand. All of them were identified by comparison with the specimens at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimens of *Terminalia bellerica* (SRU 021), *Terminalia chebula* (SRU 022), and *Phyllanthus emblica* (SRU 023) were deposited at Faculty of Oriental Medicine, Rangsit University, Pathumtani, Thailand. The Tri-phala preparation was formulated by using the equal amount of *Terminalia bellerica, Terminalia chebula* and *Phyllanthus emblica*.

***Preparation of crude extracts*** The Tri-phala preparation was formulated by using the equal amount of 500 g of *Terminalia bellerica, Terminalia chebula* and *Phyllanthus emblica*. Then the mixture was boiled in water (3 L) and let the water evaporate freely to obtain 1 liter of the aqueous extract. Next, the water was removed from the extract by using freeze dry method.

***Preparation of standard solutions*** Stock standard solution was prepared over the range of concentrations expressed in ng/spot by making the measurements at 6 concentrations. The standard solutions were spotted on the TLC plate to obtain final concentrations at 440, 880, 1320, 1760, 2200 and 2640 ng/spot.

***Preparation of sample solution***

Tri-phala effervescent tablet composed of Tri-phala extract 25%, sodium bicarbonate, citric acid, tartaric acid, PEG 6000, and sodium saccharin. The effervescent tablet (4000 mg) was accurately weighed and transferred to a 100 mL beaker which filled with water (45 mL). The effervescent tablet was disintegrated completely in a solution within 5 minutes. Then the solution was transferred to volumetric flask 50 mL and adjusted to volume with water. The mixture was mixed well and filtered through membrane filter 0.45 µm to give clear sample solution.

***Validation of the Method*** The method was validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) according to the International Conference of Harmonization (ICH) guidelines.

(a) *Linearity*-- Linearity relationship between peak area and concentration of the standard solutions was evaluated at 6 concentration levels over the range of 440-2640 ng/spot. Each concentration was done in triplicate.

(b) *Precision*-- Repeatability and intermediate precision of the developed method were expressed in term of relative standard deviation (RSD) of peak area. Intraday precision was studied by repeat analyzing on the same day at three concentrations (440, 880 and 1320 ng/spot) of standard gallic acid solution (n=3). Interday precision was determined on three different days at the same three concentrations by the proposed method.

(c) *Accuracy*-- The accuracy of the method was evaluated by performing recovery studies by adding known amount of the reference compound at three levels (424, 636 and 848 ng/spot) to the effervescent tablet sample solution. Then the solutions were applied on TLC plate and conducted chromatography. Three determinations were performed for each level of concentration and the recoveries were calculated.

(d) *Specificity*--Peak purity of gallic acid was assessed by scanning the standard and sample spots. Correlation coefficients of these spectra were calculated.

(e) *LOD and LOQ*-- LOD and LOQ were determined by scanning the blank (methanol) spot and detecting the noise. A series of concentrations of gallic acid standard solution (10-100 ng/spot) were spotted on the TLC plate. Signal-to-noise ratios of 3:1 and 10:1 were considered as LOD and LOQ, respectively.

***Chromatographic condition for quantitative analysis of gallic acid in the Tri-phala extract and effervescent tablet sample solutions*** Standard and sample were applied on precoated aluminium-backed HPTLC silica gel 60 $GF_{254}$ plate using Linomat 5. Each sample solution (3 µL) was applied in triplicate as narrow bands of 6 mm length using a nitrogen aspirator. The constant application rate of 150 nL/s, 5.0 x 0.45 mm densitometer slit dimension, and 20 mm/s scanning speed were used. The mobile phase consisted of dichloromethane : ethyl acetate : formic acid (40 : 56 : 4, v/v). Linear ascending development was performed in a twin-trough glass chamber saturated with 50 mL of the mobile phase for a plate. The optimized chamber saturation time for the mobile phase was 25 min at room temperature (25 ± 2 ºC). The distance covered by the solvent font was 8 cm, which took about 20 min for each run. The spots were scanned using the TLC scanner 3 in the reflectance-absorbance mode at 280 nm, operated by winCATS software. The amount of gallic acid in the samples was calculated using the calibration graph.

**RESULTS AND DISCUSSION**

In this study, we developed a suitable method for the analysis of gallic acid in Tri-phala extract and effervescent tablet. Gallic acid is main chemical constituent in *Terminalia bellerica, Terminalia chebula* and *Phyllanthus emblica* which are the major components in the extract and effervescent tablet. For the chromatographic condition, several trials were made by using different solvent systems containing non-polar solvents and relative polar solvent; dichloromethane : ethyl acetate : formic acid (40 : 56 : 4, v/v) gave the best separation. The $R_f$ value of gallic acid was 0.34 ± 0.01 and separated well from other compounds as shown in Figure 1. The UV absorption spectrums of standard and sample have been overlaid to confirm the identity and peak purity correlation coefficient was over 0.9996 (Figure 2(a)). The method validation was composed of linearity, accuracy, precision, LOD and LOQ. For linearity of gallic acid, peak areas were found to have good linear relationship with the concentration with a correlation coefficient ($r^2$) of 0.9936 and within the concentration range of 440-2640 ng/spot (Figure 2(b)). The correlation coefficient and regression equation are presented in Table 1. The interday and intraday precisions of gallic acid were performed and the results showed acceptable precision at three different concentration levels of 440 – 1320 ng/spot with RSD <2.42%. The percentage recovery at three levels of gallic acid was 101.7, 100.7 and 98.4%, with an average of 101.4% (Table 2).

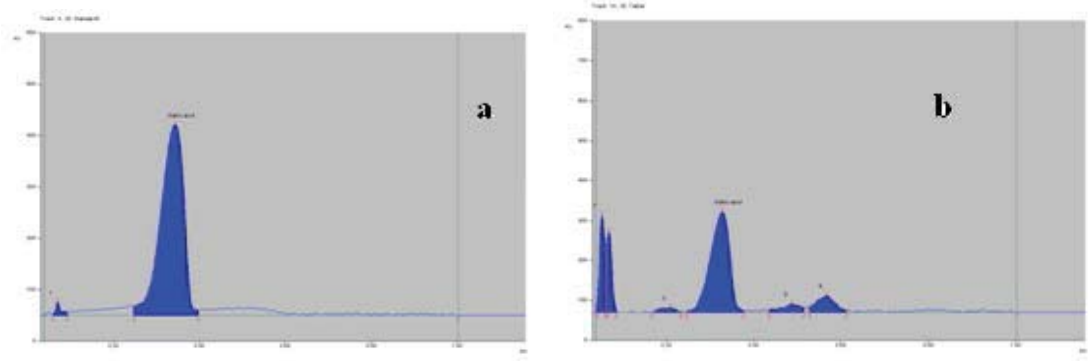


**Figure 1** (a) Standard gallic acid (b) Gallic acid in Tri-phala effervescent tablet.

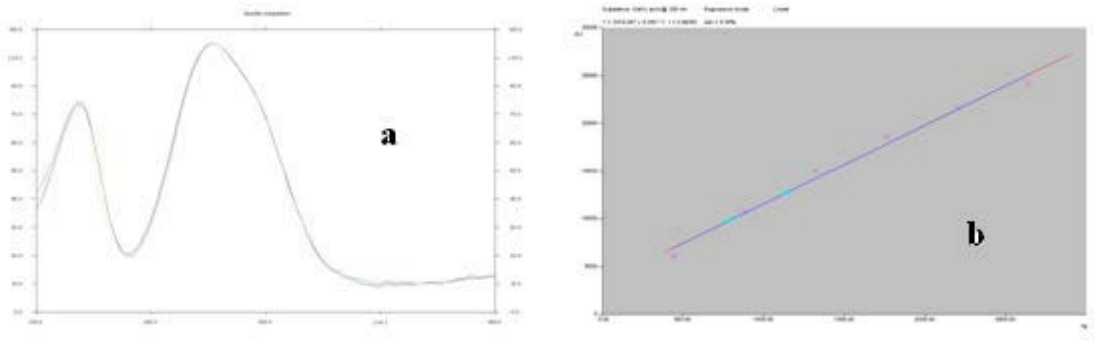


**Figure 2** (a) The UV absorption spectrums of standard gallic acid and Tri-phala sample were overlaid from 200-400 nm. (b) Calibration curve of standard gallic acid.



**Table 1** Summary of linear regression data for calibration curve of gallic acid

| Parameters | Results |
|---|---|
| Linear range | 440 – 2640 ng/spot |
| Linear regression equation | $y = 3319.267 + 8.250x$[a] |
| Correlation coefficient ($r^2$) | 0.9936 |
| Limit of quantitation, ng | 40 |
| Limit of detection, ng | 10 |
| Peak purity (specificity) | r (s,m) 0.9996, r (m,e) 0.9997 |

[a] x is the amount of gallic acid in ng, y is the peak area at 280 nm

**Table 2** Recovery study of gallic acid

| Serial No | Amount added, ng | Amount found, ng | Recovery[a,b], % |
|---|---|---|---|
| 1 | 424 | 431.06 ± 7.51 | 101.70 ± 1.77 |
| 2 | 636 | 640.78 ± 14.70 | 100.70 ± 2.31 |
| 3 | 848 | 834.42 ± 7.54 | 98.40 ± 0.89 |

[a] Expressed as mean standard deviation (SD, n=3), [b] Average recovery = 101.4%

The validated method was used in determination of gallic acid content in Tri-phala freeze dry extract and effervescent tablet (Table 3). The results showed that there were significantly different in the content (w/w) of gallic acid in both samples. The amount of gallic acid in freeze dry extract ranged from 3.30 - 3.38% (w/w) in dried powder while in the effervescent tablet ranged from 1.36 – 1.88% (w/w). The freeze dry extracts showed higher amount of gallic acid than the effervescent tablets. The reason for this issue could be subject to the preparation process to make Tri-phala effervescent tablet which used hot-air oven to dry the granule. This process might reduce the amount of gallic acid because there is a report about gallic acid can rapidly decompose at high temperatures between 105-150 ºC[7].

**Table 3** Yields of gallic acid in Tri-phala freeze dry extracts and effervescent tablets.

| Samples | Yield of gallic acid, % (w/w)[a] |
|---|---|
| Freeze dry extracts | 3.30±0.03 - 3.38±0.03 |
| Effervescent tablets | 1.36±0.02 – 1.88±0.06 |

[a] Experssed as mean standard deviation (SD, n=3)

## CONCLUSION

The HPTLC method was developed and validated for qualitative and quantitative analysis of gallic acid in Tri-phala extract and effervescent tablet. This method is simple, precise, and accurate. It can be used for quantitative analysis of gallic acid, an active anti-oxidation component in the raw materials, extracts of *Terminalia bellerica, Terminalia chebula,* and *Phyllanthus emblica* and Tri-phala effervescent tablet. This

study can be a good start for preparing traditional formulations into modern dosage forms which are easy to use and take them with us in any places.

**ACKNOWLEDGMENTS**
This work was supported by Faculty of Oriental Medicine Graduate School Program, Rangsit University, Pathumthani 12000, Thailand

**REFERENCES**

1. Bag, A., Kumar Bhattacharyya, H., Kumar Pal, N., Rajan Chattopadhyay, R. 2013. Anti-inflammatory, anti-lipid peroxidative, antioxidant and membrane stabilizing activities of hydroalcoholic extract of Terminalia chebula fruits. Pharm. Bio. Sep 5 [Epub ahed of print].
2. Pual, WH., Kim, SH. 2013. MAPK inhibitors augment gallic acid-induced A549 lung cancer cell death through the enhancement of glutathione depletion. Oncol. Rep. 30(1):513-519.
3. วุฒิ วุฒิธรรมเวช. สารานุกรมสมุนไพร. กรุงเทพมหานคร:สำนักพิมพ์โอเดียนสโตร์, 2540.
4. Pallti, F., bruni, R., Righi, D., Grandini, A., Tognolini, M., Pio Prencipe, F., Poli, F., Benvenuti, S., Del Rio, D., Rossi, D. 2013. Metabolite profiling of polyphenols in a Terminalia chebula Retzius ayurvedic decoction and evaluation of its chemopreventive activity. J. Ethnopharmacol. 147(2):277-85.
5. Jadon, A., Bhadauria., Shukla, S. 2007. Protective effect of erminalia belerica Roxb. And gallic acid against carbon tetrachloride induce damage in albino rats. J. Ethnopharmacol. 109(2):214-8.
6. Yang, B., Kortesniemi, M., Liu, P., Karonen, M., Salminen, J.P. 2012. Analysis of hydrolysable tannins and other phenolic compounds in emblic leafflower(Phyllanthus emblica L.) fruits by high performance liquid chromatography-electrospray ionization mass spectrometry. J. Agri. Food. Chem. 60(35):8672-83.
7. Boles JS, Crerar DA, Grissom G, Key TC. 1998. Aqueous thermal degradation of gallic acid. Geochim. Cosmochim. Ac. 52(2):341-344.