Marker analysis of polyherbal formulation, *Triphala* – A well known Indian traditional medicine

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Triphala is one of the ages old; most commonly used polyherbal preparation in Indian System of Medicine (ISM) particularly in Ayurveda. A rapid, simple, and accurate method with high performance thin layer chromatography (HPTLC) has been developed to standardize *Triphala* and its individual component using gallic acid (GA) as analytical marker compound. Methanol extracts of *Triphala*, *Emblica officinalis*, *Terminalia chebula* and *Terminalia belerica* were used for HPTLC on silica gel plates. The R_f of GA was found to be 0.80 with densitometric scanning at 254 nm and the calibration plot was linear in the range of 400 ng to 1800 ng of GA. The correlation coefficient, 0.999, was indicative of good linear dependence of peak area on concentration. The GA content in *Triphala* with its individual constituents like *Emblica officinalis*, *Terminalia chebula* and *Terminalia belerica*, was found to be 14.38, 17.50, 16.60 and 11.92 mg g^{-1}. This method permits reliable quantification of GA with good resolution and separation of the same from other constituents of extracts of *Triphala* and its constituents. Recovery values from 96.86 to 98.71% showed the reliability and reproducibility of the method. The proposed HPTLC method for quantitative monitoring of GA in *Triphala* and its constituents can be used for routine quality testing and similar methods can be developed for other herbal formulations.

Keywords: HPTLC, Standardization, Triphala, Gallic acid, Ayurveda, ISM

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Identification and quality evaluation of crude drugs is a fundamental requirement of Industry and other organizations dealing with natural health products (NHP). The fact must be taken into account that the plant material to be examined has a complex and inconsistent compositions based on its contents of secondary metabolites. Therefore, the analytical limits are not as precise as for the single chemical entity[1]. It is an accepted fact that qualitative and quantitative analysis of major bioactive chemical components (marker components) of crude drug constitute an important and reliable part of quality control protocol as any change in quality of the drug directly affects the constituents[2,3]. *Triphala* is one of the well known powdered preparations in Indian System of Medicine, being used in Ayurveda since ancient time. This well-known phytomedicine is made in combination with *Terminalia chebula, Terminalia belerica* and *Emblica officinalis*, in equal proportions as reported in the Ayurvedic Formulary of India (AFI)[4]. This formulation is prescribed in the first line treatment

of many aliments as laxative, detoxifying agent and rejuvenator in Ayurveda. Its antidiabetic, antimutagenic, purgative and radio protective activities has been reported[5-9]. The individual herbs, used in the formulation are reported to have several other health benefits. *Emblica officinalis* is reported to possess antiinflammatory, antimutagenic, antioxidant, cytoprotective, gastroprotective, hypolipidaemic activity[7,10-14]. Similarly, *Terminalia chebula* possess antibacterial, anticancer, anticaries, antimutagenic potential and inhibits local anaphylazis[15-19]. *Terminalia belerica* is reported to protect myocardial necrosis, reduces cholesterol-induced atherosclerosis and acts as hepatoprotective[20-22].

Gallic acid (GA) is a common phyto-constituent present in all the three herbs used in *Triphala* and it is reported to possess hepatoprotective and antioxidant activity, so the quantification of GA can be helpful in routine quality control of *Triphala* and its different constituents[22-24]. To develop marker analysis for this Ayurvedic formulation, a HPTLC method for standardization of the extracts of *Triphala* and its

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individual components using GA as marker compound has been made. The HPTLC method described here is rapid, precise, sensitive, and reproducible and gives good recovery for standardization of *Triphala* and its constituents. Details on the quantitative evaluation of GA in *Triphala* and its various constituents are being reported.

Methodology

The plant materials {fruits of *Terminalia chebula* Retz. (Combretaceae), *Terminalia belerica* Linn. (Combretaceae) and *Emblica officinalis* Gaertn. (Euphorbiaceae)} were purchased from local market and authenticated at Botanical Survey of India, Shibpur, West Bengal. A voucher specimen is preserved in the laboratory for further reference. Seeds from individual fruits were removed and the dried fruits pulps were crushed to powder using grinder. *Triphala* was prepared from these powders by mixing them in equal proportions (1:1:1) based on formula of Ayurvedic Formulary of India[4]. These powders were stored in a closed vessel for further use. GA standard was obtained from Sigma Aldrich, St Louis, USA. Analytical grade toluene, ethyl acetate, glacial acetic acid, formic acid and methanol were obtained from E Merck, Mumbai. *Triphala* and powders of each plant sample (1 gm) were extracted with methanol (90 ml) for 6 hrs in a Soxhlet apparatus. The extract was filtered through Whatman (Maidstone, England) filter paper No1, pore size 1μm, and final volume was adjusted to 100 ml with methanol in a volumetric flask. 10 ml of these solutions were evaporated to dryness on a water bath and once again dissolved in 2 ml of methanol. These solutions were used for the HPTLC analysis.

A Camag (Muttenz, Switzerland) HPTLC system made up of a Linomat IV sample applicator, a twin trough plate development chamber, TLC Scanner 3 and winCATS integration software was used. Aluminum backed HPTLC plates 20 × 20 cm with 0.2 mm layers of silica gel 60 F_{254} (E Merck, Mumbai), previously pre-washed with methanol was used. GA was used as reference standard with which a working standard (100 μg ml^{-1}) was prepared, 2-20 μl of this solution was applied by means of a Camag Linomat IV sample applicator to the plates about 1 cm above the edge using a bandwidth of 5 mm and distance between tracks of 5 mm. The chromatogram was developed up to 80 mm under chamber saturation conditions with toluene: glacial acetic acid: formic acid (20:45:20:05) in a Camag twin trough chamber. After development and drying, the plate was observed under UV light for the presence GA, which was detected by a prominent dark brown colour spot. The plate was then scanned at Camag TLC scanner 3 at $^{\lambda}$max 254 nm. Different concentrations of GA were plotted against peak area to determine the linearity. Extract solutions were also prepared as above, 10 μl of the extract solution was applied along with 10 μl of standard solution (100 μg ml-1) in the same plate and it was developed with the same solvent system and the plates were scanned at $^{\lambda}$max 254 nm in a Camag TLC scanner 3 and the amount of GA was determined by means of the calibration plot. To study the accuracy and precision of the method, recovery studies were performed by the method of standard addition. The recovery of added standard was studied at three different levels with *Triphala*, each being analyzed in a manner similar to that described for the assay. The content of GA was quantified by the proposed method and the percentage recovery was calculated. To ascertain the effectiveness of the method, a suitability test was performed with freshly prepared standard stock solution of GA in accordance with USP protocol[25].

Results and discussion

Directives on the analytical control of crude drugs must take in to account the fact that the material to be examined has a complex and inconsistent compositions, therefore the analytical limits are not as precise as for the single chemical entity base on chemo-profiling through marker analysis[26,27]. Biomarker profiling of maximum number of medicinal plants and poly herbal formulation being used in therapy required to be established to highlight the quality control development based on this new emerging techniques which is being utilized by the people through out the globe for drug development from natural resources[3].

The method of sample preparation and the development of a suitable mobile phase are two important steps in devising an analytical procedure. With respect to the herbal drugs, such steps are especially significant because of the complexity of the chemical composition and the affinities of the components towards various solvents. By testing different mobile phases for the separation of extracts of *Triphala* and its constituents by HPTLC, the

desired resolution of gallic acid (GA) with symmetrical and reproducible peaks was achieved using toluene: ethyl acetate: glacial acetic acid: formic acid (20:45:20:05). HPTLC chromatogram of methanol extract of *Emblica officinalis*, *Terminalia chebula, Terminalia belerica* and *Triphala*, together with the reference standard (GA) has been described (Figs 1-5); the R_f of GA was 0.80. To ascertain the purity of the peak in test samples, its *in situ* reflectance spectrum was compared with that of standard GA and found to be superimposable, thus confirming the peak purity. Calibration plots of peak area against concentration were linear in the range of 400 ng to 1800 ng of GA. The calibration lines were represented by the liner equation Y=851.75 X + 14.82, with a correlation coefficient of 0.999, where X is the response for peak area and Y is for the concentration, with correlation coefficient, 0.999, was indicative of good linear dependence of peak area on concentration. The recovery values obtained were in the range of 97.08 to 98.71% for *Triphala* extract,

showing the reliability and reproducibility of the method (Table 1). The GA content of the formulation and the individual constituent was determined from the calibration plot made with the standard sample of GA. GA content in *Triphala* with its individual constituents like *Emblica officinalis, Terminalia chebula* and *Terminalia belerica*, was found to be 14.38, 17.50, 16.60 and 11.92 mg g-1, respectively. The concentrations of gallic acid in various constituents of *Triphala*, obtained were in accordance with the values reported for the same, i.e. – 2% w/w [23].

Thus, this described method allows reliable quantification of gallic acid (GA) with good resolution form other constituents of *Triphala*. The proposed HPTLC method is rapid, simple, low cost and accurate, which can be utilized for the quantitative monitoring of gallic acid (GA) in *Triphala* and its constituents, where no other accurate method is available for routine analysis and quantification of gallic acid in *Triphala* is available.

Table 1—Recovery of gallic acid from *Triphala*

Sample	Amount of sample [µL]	Amount of GA present in A [µg]	Amount of GA added in A [µg]	Amount of GA present (B + C) [µg]	Total GA found [µg]	Recovery [%][a]
	A	B	C	D	E	F
1	10	7.19	10	17.19	16.82	97.86
2	10	7.19	20	27.19	26.84	98.71
3	10	7.19	30	37.19	36.10	97.08

[a]F=E/D × 100; average percentage recovery=97.88%
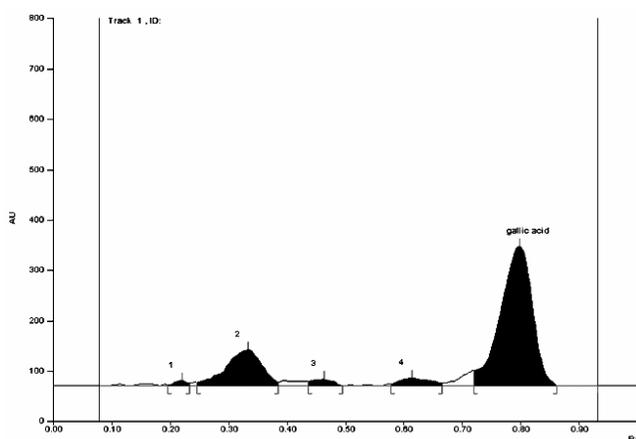
Fig. 1—HPTLC chromatogram of *Emblica officinalis*
methanolic extract
[peak 5 represents gallic acid (GA)]
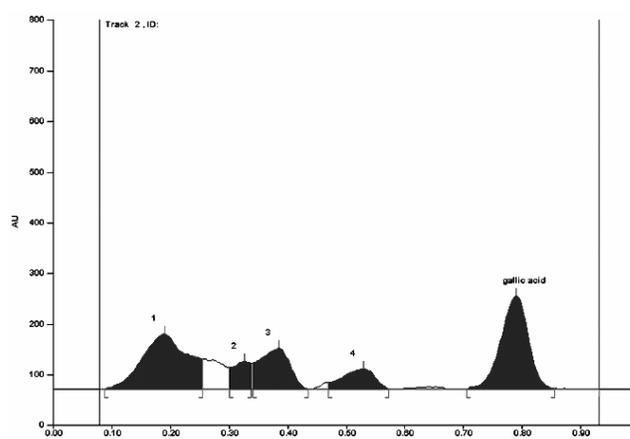
Fig. 2—HPTLC chromatogram of *Terminalia chebula*
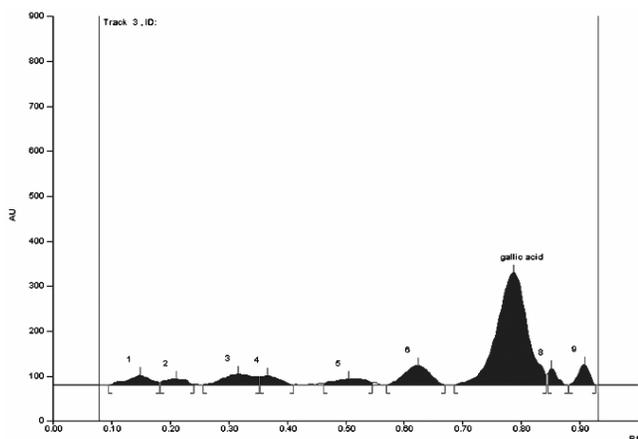methanolic extract
[peak 5 represents gallic acid (GA)]

Fig. 3—HPTLC chromatogram of *Terminalia belerica*
methanolic extract
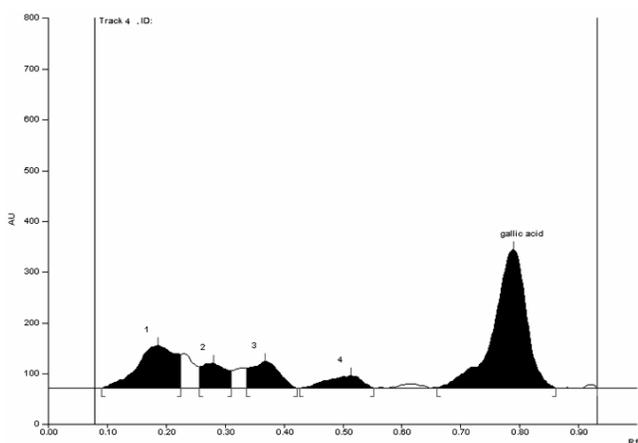[peak 7 represents gallic acid (GA)]

Fig. 4—HPTLC chromatogram of *Triphala* methanolic extract
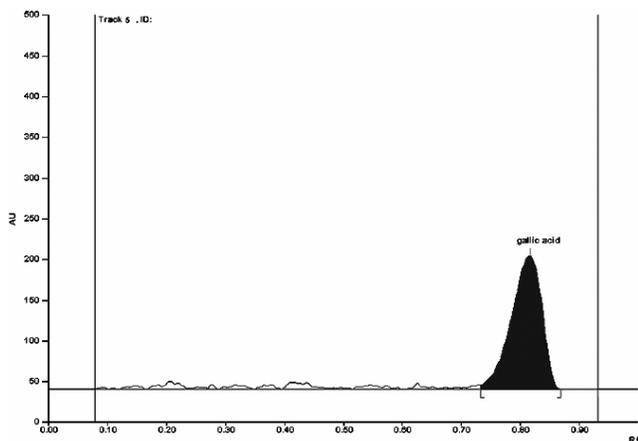[peak 5 represents gallic acid (GA)]

Fig. 5—HPTLC chromatogram of standard gallic acid (GA)

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