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QUANTITATIVE ESTIMATION OF CATECHIN, QUERCETIN AND β-CAROTENE FROM POLYHERBAL FORMULATION

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Keywords:

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ABSTRACT: An HPTLC method was developed for qualitative and quantitative estimation of catechin, quercetin and β-carotene in the marketed and in house polyherbal formulation Maharishi Amrit Kalash (MAK-5). The method was developed using precoated HPTLC silica gel 60 F254 as stationary phase and toluene: ethyl acetate: formic acid (12:12:2, v/v/v) as mobile phase for catechin and quercetin and petroleum ether (40-60^{0}C): acetone (17:3, v/v) for β-carotene. Detection and quantification were performed densitometrically at 200nm for catechin and quercetin and 430 nm for β-carotene. The Rf values of catechin, quercetin and β-carotene are 0.29 ± 0.01, 0.49 ± 0.01 and 0.70 ± 0.01 respectively. The percentage content of catechin, quercetin and β-carotene in marketed and in house polyherbal formulation are 0.223%w/w, 0.099%w/w and 2.313%w/w and 0.114%w/w, 0.141%w/w and 2.486%w/w respectively. Linearity was observed in the concentration range of 100-800 ng/spot for catechin and quercetin and that of 100-600ng/spot for β-carotene. Quantification of catechin, quercetin and β-carotene was found to be achieved by HPTLC fingerprinting from marketed and inhouse polyherbal formulation.

INTRODUCTION: High performance thin layer chromatography (HPTLC) is emerging as a versatile, high-throughput, and cost effective technology that is uniquely suited to assessing the identity and quality of botanical materials. HPTLC analysis of medicinal plants is a great contribution to the plant- drug analysis and other such botanical pharmacognosy works of modern times.

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It is an ideal tool for identification tests as it is faster, easier and more flexible. A strong point of HPTLC is the large number of samples that can be analyzed in parallel, affording rapid results, which can be conveniently compared. HPTLC fingerprints are often employed during product and process development to establish proper extraction parameters, to standardize or normalize extracts, and to detect any changes or degradations in the material during formulation [1-3].

HPTLC is an analytical technique based on TLC, but with enhancements intended to increase the resolution of the compounds to be separated and to allow quantitative analysis of the compounds [4]. HPTLC offers many advantages over other

chromatographic techniques such as unsurpassed flexibility (esp. stationary and mobile phase), choice of detection, user friendly, rapid and cost effective. Quantitative HPTLC determination of active constituents or markers of botanical drugs is performed by scanning densitometry. In the present study HPTLC fingerprinting helps us for qualitative and quantitative determination of standards or biomarkers in polyherbal formulation.

Maharishi Amrit Kalash (MAK) belongs to a group of polyherbal formulations called "Rasayanas" [5]. MAK enhances immunity, optimizes physiological balance (homeostasis), counters the degenerative effects of ageing and promotes health and longevity. MAK enhances immunity and prevents free radical generation [6]. Maharishi Amrit Kalash is presented in two forms: a paste called MAK-4 made up of thirty-eight herbs lipophilised in cow ghee and a hydrophilic tablet known as MAK-5 composed of thirteen herbs. The present study was taken up for qualitative and quantitative estimation of catechin, quercetin and β-carotene from marketed an inhouse polyherbal formulation.

MATERIALS AND METHODS:

Chemicals and reagents:

Standards catechin, quercetin and β-carotene were obtained from Sigma Aldrich chemicals. All chemicals used were of analytical grade and obtained from Merck chemicals. Marketed formulation Maharishi Amrit Kalash comprising of MAK-4 and MAK-5 was bought from Gala Ayurvedic Bhandar, Mulund, Mumbai, India.

Equipment:

A Camag HPTLC system comprising of Camag linomat V automatic sample applicator, twin trough development chamber, Hamilton syringe (100ul), Camag TLC scanner-IV, Win CATS software version 1.4.6, Camag reprostar-3, Camag TLC plate heater were used during the study.

Plant material:

Polyherbal formulation consists of 13 ingredients viz. *Withania somnifera, Glycirrhiza glabra, Ipomoea digitata, Asparagus adscendens, Emblica officinalis, Tinospora cordifolia, Asparagus racemosus, Vitex trifolia, Convolvulus pluricaulis, Argyreia speciosa, Curculigo orchioides, Capparis aphylla* and *Acacia Arabica*. All these plant parts were procured from the local market of Mumbai, Pune and nearby forest areas and were authenticated by Blatter herbarium, Mumbai and Sunrise agro services, Pune depending on the availability of plants.

Preparation of inhouse polyherbal formulation:

All the ingredients were collected, dried and powdered separately, passed through the 45# sieve and then mixed together in specified proportions in geometrical manner to get uniform mixture.

Preparation of standard solution Catechin, Quercetin and β-carotene:

Preparation of standard Catechin:

5 mg of weighed quantity of catechin was dissolved and diluted to 5ml of methanol. From that 1ml of solution was pipetted out and diluted to 10 ml of methanol (100ng/µl).

Preparation of standard Quercetin:

5 mg of weighed quantity of quercetin was dissolved and diluted to 5ml of methanol. From that 1ml of solution was pipetted out and diluted to 10 ml of methanol (100ng/µl).

Preparation of standard β-carotene:

5 mg of weighed quantity of β-carotene was dissolved and diluted to 5ml of methanol. From that 1ml of solution was pipetted out and diluted to 10 ml of methanol (100ng/µl).

Preparation of sample solution:

0.1g each of marketed formulation and inhouse formulation was mixed with hydroalcoholic solution (distilled water: methanol, 1:1 v/v) and 4-5 drops of conc. HCl added in 125cm^3 glass stoppered conical flask separately. Both flasks were sonicated using ultrasound sonicator [7] for around 2 hours with regular intervals. The extract was filtered through Whatmann filter paper no. 1 and volume was made upto 10ml with hydroalcoholic solution (1:1 v/v) and final concentration was 10µg/µl. It was further used for HPTLC.

Development of HPTLC technique:

The sample solutions were spotted in the form of bands of width 8.0 mm with a Camag microlitre syringe on precoated silica gel aluminium plate

60F254 (20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat V (Switzerland). The plates were prewashed by methanol and activated at 120°C for 20 min prior to chromatography. The slit dimension was kept at 6.0mm × 0.45 mm and 20 mm/s scanning speed was employed. Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with filter paper whatman no. 1 in the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature (25^0 C ± 2) at relative humidity of 60% ± 5.

The length of chromatogram run was 8.0 cm. Subsequent to the scanning, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed with Camag TLC scanner IV in the reflectance absorbance mode at 200 nm and operated by Win CATS software (1.4.6 Camag) with the help of D2 lamp for catechin and quercetin. Tungsten lamp at 430 nm was used for β-carotene. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was carried out by comparing peak areas with linear regression.

RESULTS AND DISCUSSION:

Catechin and Quercetin:

The mobile phase consisted of toluene:ethyl acetate:formic acid, in the volume ratio of 12:12:2 (v/v/v) for catechin and quercetin. The first 9 tracks on HPTLC plate consists of standard catechin and quercetin; tracks 10, 11 and12 consist of marketed polyherbal formulation and last three tracks 13, 14 and 15 consisted of inhouse polyherbal formulation as shown in **Figure 1**. The spectral scan shows the peak areas and 3D chromatogram of standards catechin, quercetin, marketed and inhouse formulation in **Figure 2**. The Rf value of catechin and quercetin were found to be 0.29 ± 0.01 and 0.49 ± 0.01 respectively.

The percentage content of catechin and quercetin in hydroalcoholic extract of marketed and inhouse polyherbal formulation was 0.223% and 0.114% w/w and 0.099% and 0.141% w/w respectively. When the concentration and their respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r^2=0.99823 for catechin and r^2=0.99924 for quercetin) was observed. The concentration range for catechin and quercetin was 100-800 ng/spot as shown in **Figure 3** and **Figure 4**.

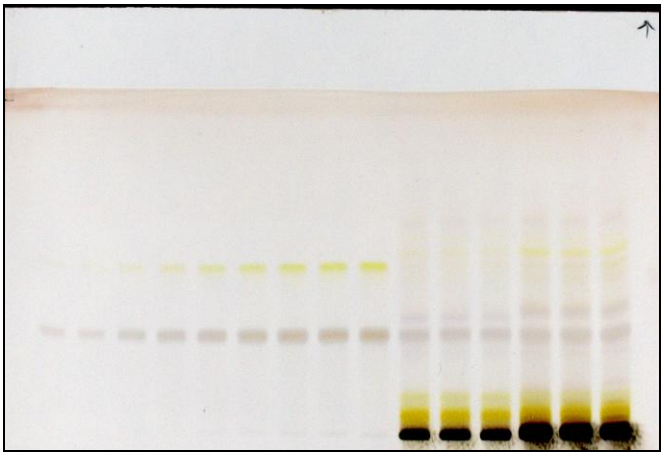
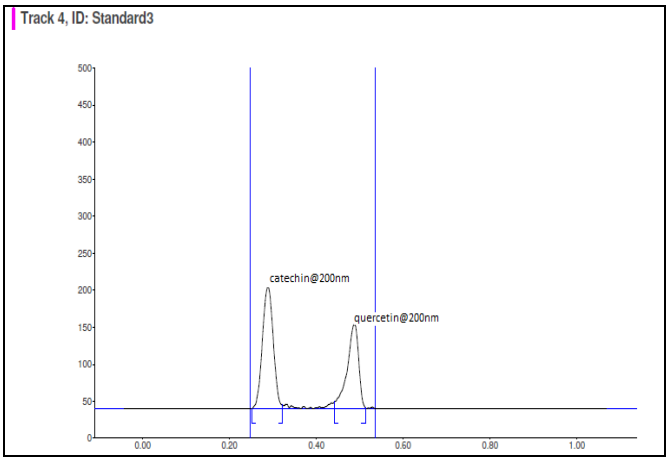
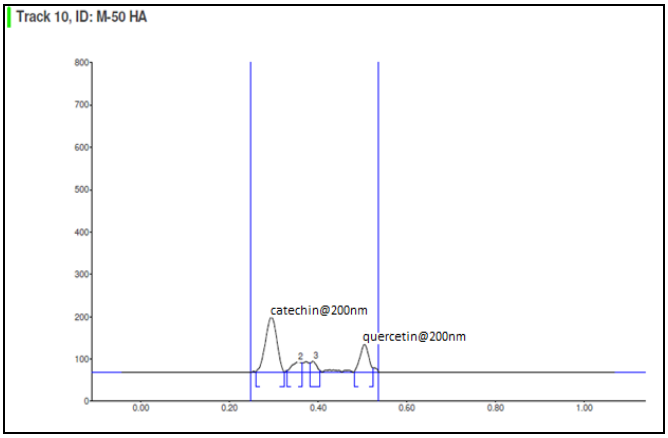


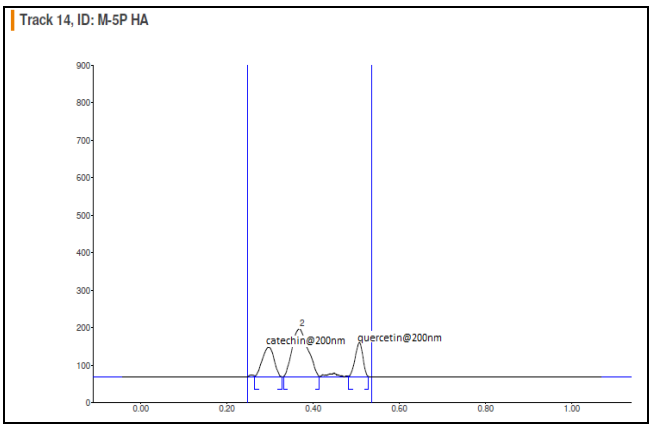
FIGURE 1: HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY SEPARATION OF STANDARD CATECHIN AND QUERCETIN AND HYDROALCOHOLIC EXTRACT OF MARKETED AND INHOUSE POLYHERBAL FORMULATION



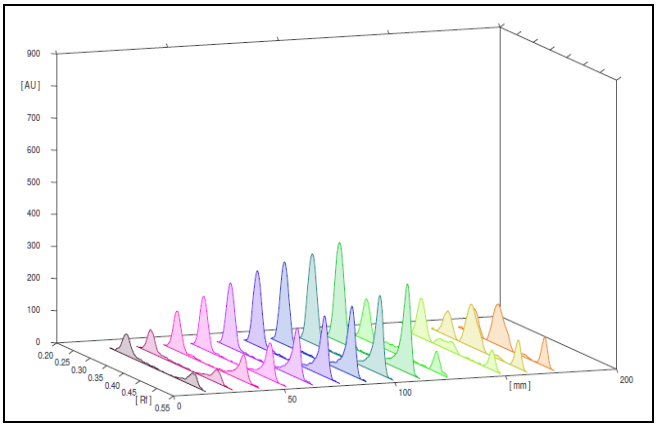
A



B



C



D

FIGURE 2: A: STANDARDS CATECHIN AND QUERCETIN B: CATECHIN AND QUERCETIN IN HYDROALCOHOLIC EXTRACT OF MARKETED FORMULATION (M-5O HA) AT 200nm; C: CATECHIN AND QUERCETIN IN HYDROALCOHOLIC EXTRACT OF INHOUSE FORMULATION (M-5P HA) AT 200nm AND D: 3D CHROMATOGRAM OF STANDARDS CATECHIN, QUERCETIN, MARKETED AND INHOUSE FORMULATION

The regression equation for catechin was found to be Y = 791.6 + 8.532 * X and that of quercetin was found to be Y = 254.8 + 7.665 * X where Y is the peak area and X is the concentration of catechin and quercetin respectively. The regression equations were used to estimate the amounts of catechin and quercetin present in marketed and inhouse formulation. Subsequent to the development; TLC plate containing catechin and quercetin was dipped in Anisaldehyde sulphuric acid reagent followed by drying on electric plate heater at 110^0 C.

β-carotene:

The mobile phase consisted of petroleum ether (40-60^0C): acetone, in the volume ratio of 17:3 (v/v) for β-carotene. The first and last three tracks consisted of 5, 10 and 15 µl of hydroalcoholic extract of marketed and inhouse polyherbal formulation respectively. The remaining tracks consisted of standard β-carotene solution (**Figure-5**). The spectral scan shows peak areas and 3D chromatogram of β-carotene, marketed and inhouse polyherbal formulation as shown in **Figure-6**.

The Rf value of β-carotene was found to be 0.70 ± 0.01. The percentage content of β-carotene in hydroalcoholic extract of marketed and inhouse polyherbal formulation was 2.313%w/w and 2.486%w/w respectively. When the concentration and their respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r^2=0.99968) was observed. The concentration range for β-carotene was 100-600 ng/spot (**Figure-7**). The regression equation for β-carotene was found to be Y=-0.1052 + 1449 * X where Y is the peak area and X is the concentration of β-carotene. The regression equations were used to estimate the amount of β-carotene in marketed and in house polyherbal formulation

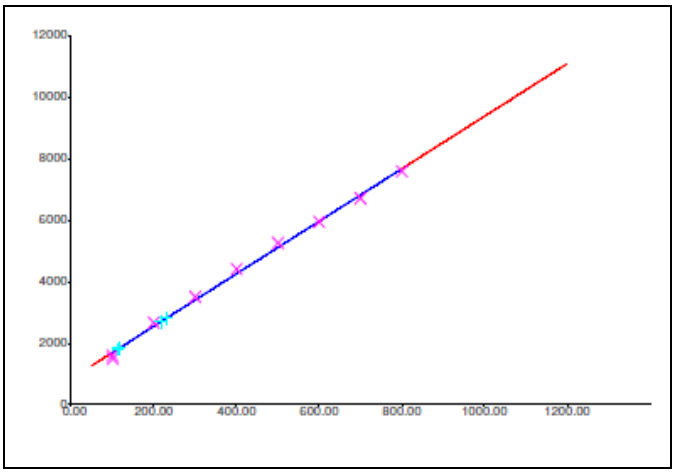


FIGURE 3: CALIBRATION CURVE FOR CATECHIN

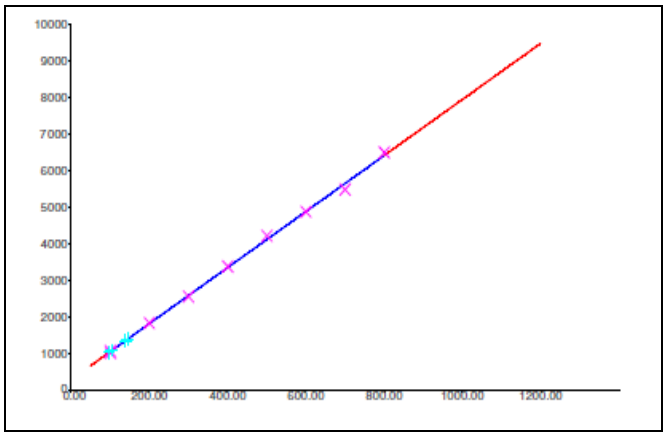


FIGURE 4: CALIBRATION CURVE FOR QUERCETIN

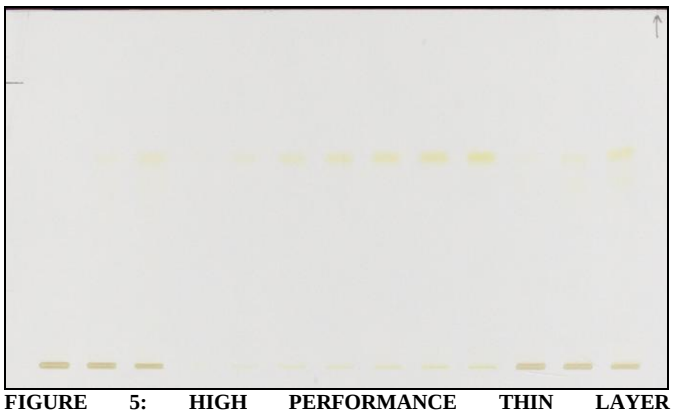
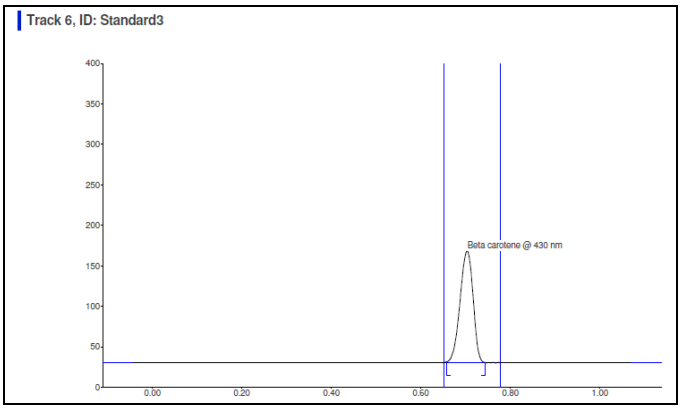
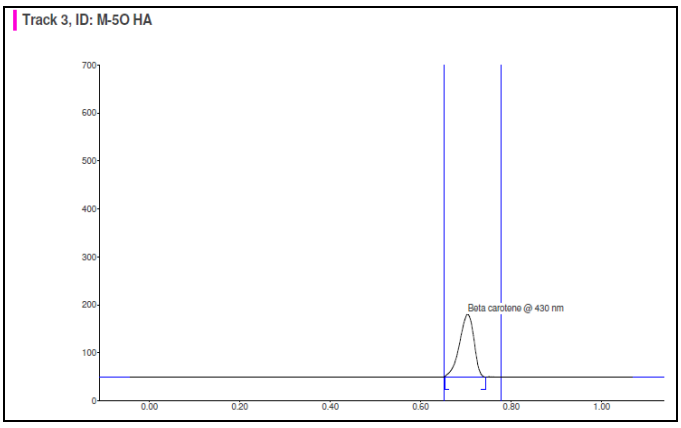


FIGURE 5: HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY SEPARATION OF STANDARD β-CAROTENE AND HYDROALCOHOLIC EXTRACT OF MARKETED AND INHOUSE POLYHERBAL FORMULATION



E



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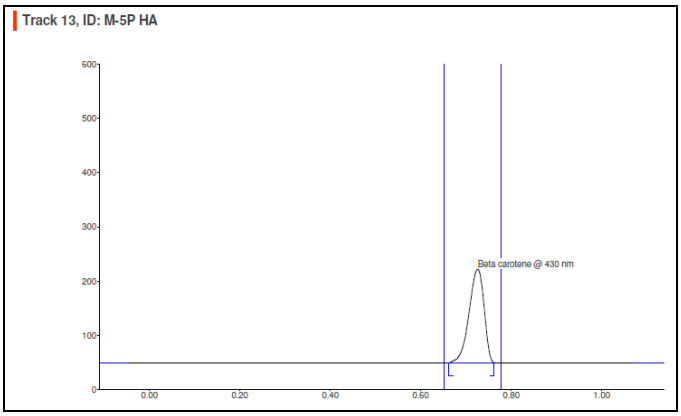
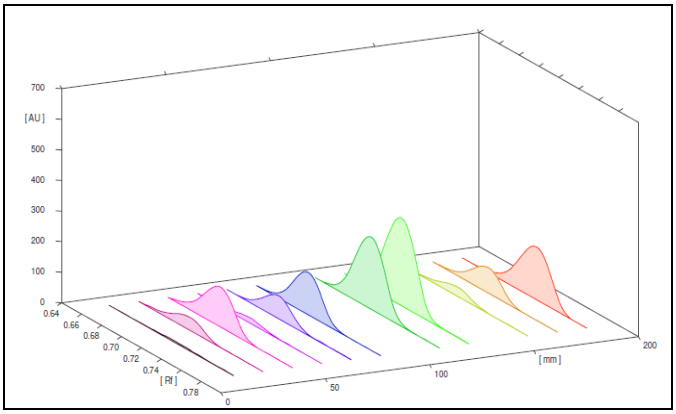


G



H

FIGURE 6: E: STANDARD β -CAROTENE F: β -CAROTENE IN HYDROALCOHOLIC EXTRACT OF MARKETED FORMULATION (M-5O HA) AT 430nm; G: β -CAROTENE IN HYDROALCOHOLIC EXTRACT OF INHOUSE FORMULATION (M-5P HA) AT 430nm AND H: 3D CHROMATOGRAM OF STANDARD β-CAROTENE, MARKETED AND INHOUSE FORMULATION

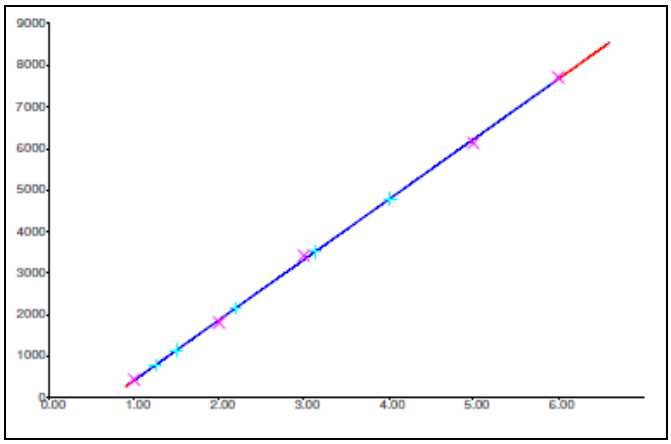


FIGURE 7: CALIBRATION CURVE FOR β-CAROTENE

CONCLUSION: HPTLC fingerprinting was used for qualitative and quantitative determination of standards or biomarkers catechin, quercetin and β-carotene from hydroalcoholic extract of marketed and inhouse polyherbal formulation Maharishi Amrit Kalash (MAK-5). This analysis of polyherbal formulation can provide standard fingerprints with selected solvent system and can be used as a reference for identification and quality control of drug.

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