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Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations

M.J. Ansari [a,*], S. Ahmad [b], K. Kohli [a], J. Ali [a], R.K. Khar [a]

[a] *Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard University, New Delhi 110062, India*
[b] *Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard University, New Delhi 110062, India*

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Abstract

A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic method of analysis of curcumin both as a bulk drug and in formulations was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of chloroform:methanol (9.25:0.75 v/v). This system was found to give compact spots for curcumin (R_f value of 0.48 ± 0.02). Densitometric analysis of curcumin was carried out in the absorbance mode at 430 nm. The linear regression analysis data for the calibration plots showed good linear relationship with $r = 0.996$ and 0.994 with respect to peak height and peak area, respectively, in the concentration range 50–300 ng per spot. The mean value ± S.D. of slope and intercept were 1.08 ± 0.01, 51.93 ± 0.54 and 8.39 ± 0.21, 311.55 ± 3.23 with respect to peak height and area, respectively. The method was validated for precision, recovery and robustness. The limits of detection and quantitation were 8 and 25 ng per spot, respectively. Curcumin was subjected to acid and alkali hydrolysis, oxidation and photodegradation. The drug undergoes degradation under acidic, basic, light and oxidation conditions. This indicates that the drug is susceptible to acid, base hydrolysis, oxidation and photo oxidation. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of said drug. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Keywords: Curcumin analysis; HPTLC method; Validation; Stability indicating

1. Introduction

Curcumin 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione (Fig. 1) is a yellow colored phenolic pigment obtained from powdered rhizome of *Curcuma longa* Linn. (Family: Zinziberaceae) [1]. It has been used to relieve the pain and inflammation since ancient times in traditional medicine. Extensive researches have revealed potent anti-inflammatory effects of curcumin [2–4]. It appears to block synthesis of certain prostaglandins [5], reduces pro-inflammatory cytokine synthesis [6,7], inhibit pro-inflammatory arachidonic acid as well as neutrophils aggregation [8,9] during inflammatory states. However, the oxygen radical scavenging activity [10,11] of curcumin has also been implicated in its anti-inflammatory effects [12]. Curcumin is unstable at basic pH and undergoes alkaline hydrolysis in alkali/higher pH solution. Hydrolytic decomposition is reported even in in vitro physiological condition (isotonic phosphate buffer, pH 7.2) [13–15]. It undergoes photodegradation when exposed to light in solution as well as in solid form [13]. Various methods are available for the analysis of curcumin in the literature like UV [16], HPLC [17–19], TLC [20,21] and HPTLC [22], but there are very few reports on analytical methods for the estimation of curcumin in bulk and its dosage form. Moreover, none of them is stability-indicating method. The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires the stress testing to be carried out

* Corresponding author. Tel.: +91 9891366489.
E-mail address: javedpharma@rediffmail.com (M.J. Ansari).

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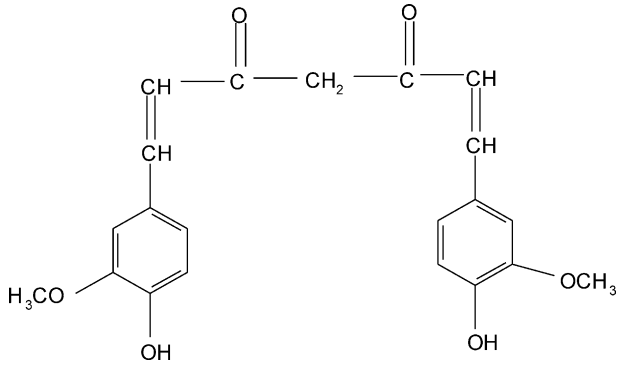

Fig. 1. Structure of curcumin.

to elucidate the inherent stability characteristics of the active substance [23]. Susceptibility to oxidation is one of the required tests. Also, the hydrolytic and the photolytic stability are required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products. Nowadays, HPTLC is becoming a routine analytical technique due to its advantages [24–26]. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. Mobile phases having pH 8 and above can be employed. Suspensions, dirty or turbid samples can be directly applied. It facilitates automated application and scanning in situ. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters. Simultaneous assay of several components in a multicomponent formulation is possible. The aim of this work is to develop an accurate, specific, repeatable and stability-indicating method for the determination of curcumin in the presence of its degradation products and related impurities as per ICH guidelines.

2. Experimental

2.1. Materials

Curcumin was purchased from Loba Chemicals Bangalore, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

2.2. HPTLC instrumentation

The samples were spotted in the form of bands of width 3 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F-254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland). A constant application rate of 150 nl/s was employed and space between two bands was 5.5 mm. The slit dimension was kept at 4 mm × 0.1 mm, and 20 mm/s scanning speed was employed. The mobile phase consisted of chloroform:methanol (9.25:0.75 v/v). Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 10 min at room temperature. The length of chromatogram run was 65 mm. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner IV in the absorbance mode at 430 nm. The source of radiation utilized was deuterium and tungsten lamp.

2.3. Calibration curves of curcumin

A stock solution of curcumin (100 μg/ml) was prepared in methanol. Different volumes of stock solution, 0.5, 1, 1.5, 2, 2.5 and 3 μl were spotted in duplicate on TLC plate to obtain concentrations of 50, 100, 150, 200, 250 and 300 ng per spot of curcumin, respectively. The data of peak height/area versus drug concentration were treated by linear least-square regression.

2.3.1. Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (100 ng per spot of curcumin). The intra- and inter-day variation for the determination of curcumin was carried out at two different concentration levels of 100 and 200 ng per spot.

2.3.2. Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of chloroform:methanol (9.5:0.5 and 9:1 v/v) was tried at two different concentration levels of 100 and 200 ng per spot.

2.3.3. Limit of detection and limit of quantification

In order to determine detection and quantification limits, curcumin concentrations in the lower part of the linear range of the calibration curve were used. Curcumin solutions of 50, 100 and 150 ng/μl were prepared and applied in triplicate (1 μl each). The amount of curcumin by spot versus average response (peak area) was graphed and the equation for this curve was determined, thereby obtaining an estimate of the target response: ybl. The ybl value corresponds to the intersection of the curve. Subsequently, a second curve was graphed showing the amount of curcumin by spot versus standard deviation of the responses. From the equation of this curve, we obtained an estimate of the standard deviation for target: sbl, which corresponds to the intersection of this curve. Detection and quantification limits were calculated by means of the equations [27]: detection limit = (ybl + 3 sbl)/*b*; quantification limit = (ybl + 10 sbl)/*b*, where "*b*" corresponds to the slope obtained in the linearity study of the method.