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Research Article

HPTLC: A Tool for Determination of Curcumin in Mammalian Samples

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ABSTRACT

Curcumin, an important phytoconstituent obtained from *Curcuma longa* L. (turmeric) is used traditionally in the treatment of various inflammatory diseases like arthritis, stroke and bowel diseases, etc. Despite its many health benefits, instability of curcumin in plasma is a major issue. The retention of curcumin in plasma must be properly evaluated in order to establish its stability in biological systems. The current study presents an HPTLC method undertaken for detection of curcumin and determination of its stability in plasma and different tissues of rats. The plasma and tissue samples were appropriately processed to render them suitable for HPTLC analysis. The method employed HPTLC glass plates precoated with silica gel $60F_{254}$ as the stationary phase. The mobile phase developed consisted of chloroform, methanol and glacial acetic acid which successfully gave distinct bands for curcumin with a R_f value of 0.95. This newly developed HPTLC method was found to be reproducible and accurate in quantifying curcumin in mammalian samples. This method was further utilized to efficiently estimate the time for which curcumin is retained in its native form in mammalian tissues and plasma alike.

Keywords: HPTLC, mammalian samples, curcumin, retention time.

INTRODUCTION

Curcumin (diferuloylmethane)-a water-insoluble polyphenol extracted from the herb *Curcuma longa L.* bearing the chemical formula $C_{21}H_{20}O_6$ and chemical structure 1, 7-bis (4- hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione has been widely used in traditional Indian and Chinese medicine as well as in the food industry[1]. This polyphenol has demonstrated several types of biological and pharmacological activities, including anti-inflammatory, anti-oxidant and anti-cancer properties, etc[2]. In addition, curcumin has been shown to slow down the progress of Alzheimer's disease by reducing β-amyloid[3] and delaying the onset of kainic acid-induced seizures[4]. It is also capable of inhibiting the formation of brain tumors[5]. Nevertheless, an obvious lacuna of curcumin happens to be its low absorption and retention leading to rapid systemic elimination[6]. Therefore, there is need for a rapid and sensitive technique for its fast and accurate determination, especially in biological samples.

So far, there exist a number of studies related to curcumin determination by HPLC (high performance liquid chromatography) which are quite sensitive and accurate[7]. However, these reported methods have certain limitations viz. complexity in sample application, its stability on stationary phase and lesser throughput of the technique. Such limitations can be conveniently overcome by employing HPTLC (high performance thin layer chromatography) which recently is being used for the analysis of various secondary metabolites, including curcumin. Moreover, to this point, no study has been reported involving the HPTLC-based detection of curcumin in mammalian physiological systems. Here, for the first ever time, we report development of a method utilizing HPTLC as a tool for detecting and estimating the retention of curcumin in mammalian tissues and plasma samples using male Sprague-Dawley (SD) rat as the animal model.

MATERIALS AND METHODS

Materials and instrumentation

Standard drug curcumin was purchased from Sigma Aldrich (USA). All the reagents and solvents used were procured from Sigma-Aldrich (USA) and were of HPLC grade. Male SD rats, weighing 180-200 g were taken from experimental animal facility, fulfilling all ethical regulations and clearance. HPTLC analysis of the extracts was carried out on a CAMAG system (Muttenz, Switzerland).

Sample Processing

Male rats weighing 180-200 g were selected and administered orally with curcumin (50mg/kg body weight). Selection of this dose was based on findings from earlier reports[8]. The rats were placed in normal laboratory conditions (ie, normal temperature and pressure) for the specified time periods and anesthesized with ketamine-xylazine mixture (8:1 ratio per kg of body weight), in order to collect blood via cardiac puncture. The blood was centrifuged at 3000 rpm for 15 min and plasma obtained was processed by adding equal volumes of 4% NaOH to make them aqueous enough for application onto HPTLC plates. The rats were perfused with cold PBS; then different tissues of the rats namely lungs, liver, brain,

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Table 1: Intraday and interday precision and accuracy values for curcumin.

Amount of standard curcumin applied (µg/spot)	Intraday precision			Interday precision		
	Amount detected (µg/spot)	Accuracy (%)	Precision (%)	Amount detected (µg/spot)	Accuracy (%)	Precision (%)
2	1.98	99.00	2.32	1.94	97.00	2.98
3	2.87	95.66	2.71	2.75	91.66	2.32
4	3.95	98.75	1.82	3.81	95.25	2.41

kidney, heart, muscle and spleen were isolated. All the tissues were homogenized (using 0.154 M KCl) and centrifuged at 3000 rpm for 15 min. Then 200 µl of each tissue homogenate was added to an equal volume of 4% NaOH and kept aside for HPTLC analysis.

HPTLC analysis for curcumin identification and retention in mammalian samples

The study was performed in three phases.

Phase 1: In this set, rats supplemented with curcumin (n=6 per group) were incubated in laboratory conditions for three time durations viz (a) 1 h (b) 3 h and (c) 6 h. The plasma samples obtained from this set were subjected to HPTLC analysis for detection of curcumin. The mobile phase was composed of chloroform, methanol and glacial acetic acid in the ratio of 7:2:0.3, modifying a method as described elsewhere[9]. Method validation of the modified method (n=3) was performed for parameters like linearity, reproducibility, precision and accuracy. The chromatographic procedure was brought about on glass backed silica gel 60 F_{254} HPTLC plate (Merck). Three different volumes-2, 3 and 4 µL of standard curcumin solution (1 mg/mL) prepared in methanol, hence corresponding to 2, 3 and 4 µg of standard curcumin (marked in **Fig. 1A** and **1B** as Std1, Std2 and Std3) and 10 µL of plasma samples were applied as 6 mm wide bands by CAMAG Linomat 5 sample applicator. The plate was then developed at room temperature in a CAMAG twin-trough vertical development chamber, previously saturated for 7 min with the mobile phase mentioned above. The migration distance was maintained at 80 mm. Following this, the plate was subjected to densitometric scanning using CAMAG Scanner 3, at wavelength of 366 nm, maintaining a scanning speed of 20 mm s^{-1} and slit dimension of 6.0 mm x 0.45 mm, with deuterium being the light source. The scanning procedure was realized using winCats software (version 1.4.4.6337)[10]. The detection of curcumin present in plasma samples was observed on the HPTLC plates in the form of bands that held similar R_f values as that of curcumin standard.

Phase 2: In this phase, plasma was collected from rats supplemented with curcumin (n=6 per group) for three time durations- (a) 0.5 h (b) 1 h and (c) 1.5 h. HPTLC analysis of the samples was performed in a manner similar to phase 1.

Phase 3: After collecting plasma from rats in phase 2, tissues viz. heart, liver, lung, brain, muscle, kidney and spleen were isolated from these rats and processed as already mentioned above. HPTLC analysis for these tissues was undertaken as described for other two phases. Similar experiment was carried out in control rats (n=6), not supplemented with curcumin.

RESULTS AND DISCUSSION

In the present study, the use of 4% NaOH solution for dissolving plasma proteins from rat plasma samples for estimation of curcumin has been put forth for the first time. To determine the retention time of curcumin in plasma, three individual time durations were selected, i.e., 1 h, 3 h and 6 h. Detection of the developed plate from phase 1 at 366 nm demonstrated that lanes containing standard curcumin (Fig. 1A) showed distinct bands with an R_f value of 0.95. The modified method was found to have significant reproducibility (n=3). The calibration curve was linear within a range of 0.5-4 µg/mL of curcumin. The values for accuracy and precision are shown in Table 1. The lane containing plasma sample from rats exposed to curcumin for 1 h showed a light band with R_f value similar to standard curcumin, whereas the other two lanes did not show any detectable bands (Fig. 1A).

The presence of curcumin in plasma sample from rats exposed for 1 h motivated for execution of phase 2 study. Scanning of the developed plate from phase 2 at 366 nm, clearly indicated that plasma drawn from rats supplemented with curcumin for 0.5 h showed a prominent presence of curcumin (Fig. 1B). The peak area of curcumin was more in 0.5 h sample than in 1 h sample, as can be verified from its respective 3D display. The quantities of curcumin detected in 0.5 h, 1 h and 1.5 h plasma samples are depicted in Table 2.

Based on these inferences, tissues from rats in phase 2 were examined for studying curcumin retention in phase 3. Post scanning results from phase 3 at a wavelength of 366 nm brought out that bands corresponding to standard curcumin were clearly detected in lanes containing tissue samples from 0.5 h exposure, where curcumin was more pronounced in kidney and liver samples (Fig. 2). Thus, it could be asserted that distribution of curcumin was more in kidney and liver than other organs which is concurrent with previous reports[11]. Moreover, the intensity of curcumin in all tissue samples tested after 0.5 h exposure was much higher in comparison to tissues isolated after 1 h and 1.5 h exposure. The quantities of curcumin detected in all the tissue samples of the three time durations are tabulated in Table 2. Control set (Fig. 2) consisting of tissue samples from rats with no supplementation of curcumin showed virtually no bands in any tissue.

CONCLUSION

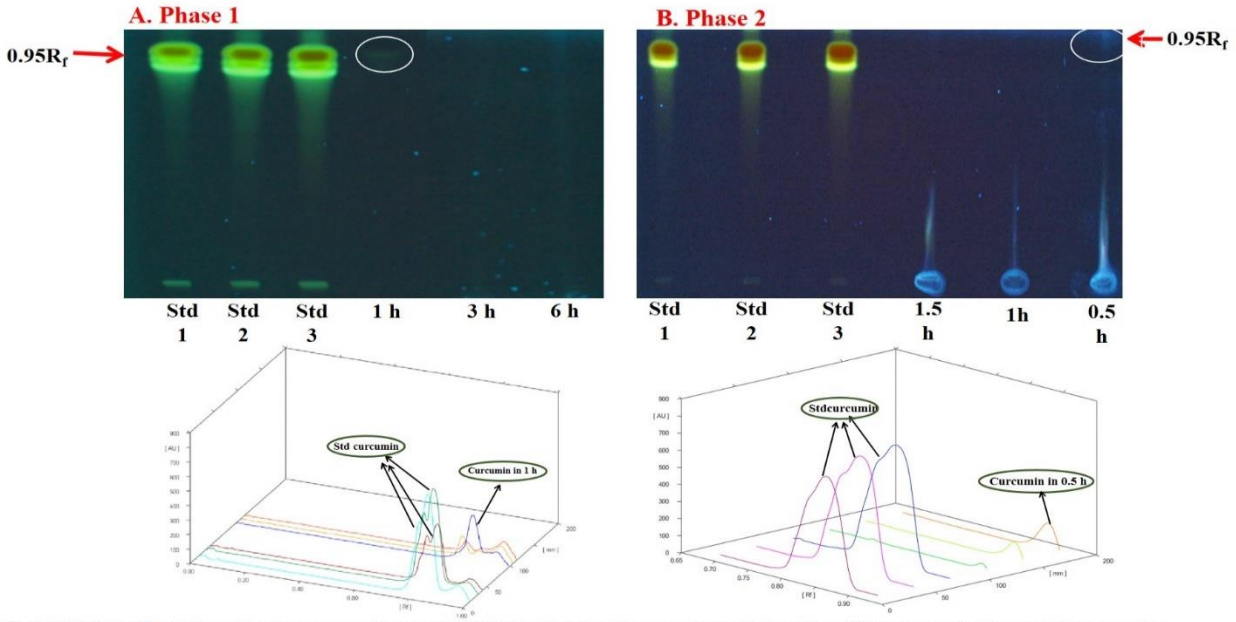

Fig.1: HPTLC profile at 366 nm showing presence of curcumin in (A) Phase I study with curcumin standards at three different concentrations and plasma samples from rats supplemented with curcumin for 1h, 3h and 6h; the circle manifests the curcumin band from plasma of 1 h sample (B) Phase II study with curcumin standards at three different concentrations and plasma samples from rats supplemented with curcumin for 1.5h, 1h and 0.5h; the circle manifests the curcumin band from plasma of 0.5h sample. The 3D figures are placed below their respective HPTLC profiles. Std- standard Curcumin

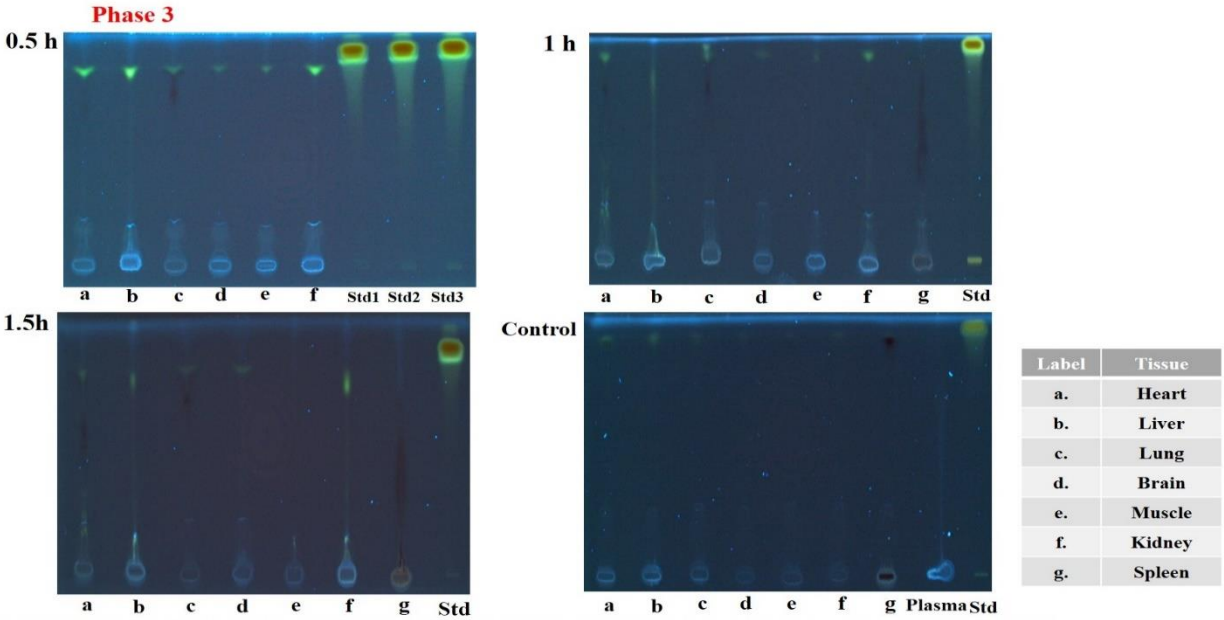

Fig.2: HPTLC profile from Phase 3 study, at 366 nm showing maximum presence of curcumin in lanes (a-g) containing tissue samples from 0.5 h as compared to 1h, 1.5 h and control samples. Std- standard Curcumin

Table 2: Quantification of curcumin in tissues and plasma of rat.

Time (in hours)	Curcumin in mg/g tissue						Curcumin in mg/mL
	HEART	LIVER	LUNG	BRAIN	MUSCLE	KIDNEY	PLASMA
0.5	1.37±0.021	2.32±0.018	1.30±0.035	1.1±0.027	1.2±0.048	1.48±0.038	0.28±0.0032
1	0.82±0.017	1.39±0.029	0.77±0.006	0.69±0.014	0.72±0.030	0.89±0.008	0.06±0.0017
1.5	0.68±0.012	1.16±0.042	0.65±0.031	0.55±0.012	0.60±0.019	0.74±0.018	Below detection limit

The current study describes a novel method for determination of curcumin in mammalian samples by HPTLC, with special focus on the optimization of sample processing for HPTLC analysis. Results substantiated that curcumin was retained in its native form in plasma and in tissues from half an hour to one hour after supplementation. Curcumin distribution majorly occurred in kidney and liver tissues up to one hour and started depleting after one hour. The developed HPTLC method was thus, established to be simple, sensitive, specific and

rapid for estimation of curcumin in plasma and different tissues of mammalian samples.

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COMPLIANCE WITH ETHICAL STANDARDS

This work was funded by Defence Research and Development Organisation, India under the project "Improving performance under different operational environments using suitable interventions". The authors declare that they have no conflict of interest. The entire study has been carried out using animal model (male SD rats). The institute's ethical committee approved all the experimental protocols and followed the guidelines of University of Federation for Animal Welfare (UFAW) for animal research.

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