New method to separation and purification of two bioactive compounds from powder of turmeric plant by RP-HPLC technique

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ABSTRACT

Two compounds were separated successfully. The used method was suitable for getting a high purified compound for each one . There is the ability to get more than one high purified compound from alcoholic extract for curcumin

Keywords: turmericplant. RP-HPLC, separation, purification

Introduction

Turmeric

Turmeric is a product of Curcuma longa, a rhizomatous herbaceous perennial plant belonging to the ginger family Zingiberoside, which is native to tropical South Asia. Asmany as 133 species of Curcuma have been identified worldwide . Most of them havecommon local names and are used for various medicinal formulations. The turmericplant needs temperatures between 20°C and 30°C and a considerable amount of annual rainfall to thrive. Individual plants grow to a height of 1 m, and have long, oblong leaves. Plants are gathered annually for their rhizomes, and are reseeded fromsome of those rhizomes in the following season. The rhizome, from which the turmeric is derived, is tuberous, with a rough and segmented Skin The rhizomes mature beneath the foliage in the ground. They are yellowish brown with a dull orangeinterior. The main rhizome is pointed or tapered at the distal end and measures 2.5–

7.0 cm (1–3 inches) in length and 2.5 cm (1 inch) in diameter, with smaller tubers branching off. When the turmeric rhizome is dried, it can be ground to a yellow powder with a bitter, slightly acrid, yet sweet, taste.

Clinical Studies Using Turmeric

Turmeric has been tested against various diseases in humans (Table 1-1). In one study, the antimutagenic effects of turmeric were examined in 16 chronic smokers (Polasa etal. 1992). Turmeric was given in doses of 1.5 g/day for 30 days, and this was found tosignificantly reduce the urinary excretion of mutagens in these smokers. In six nonsmokers, on the other hand, no change in urinary excretion of mutagens was noted. These results suggest that dietary turmeric is an effective antimutagen and maybe useful in chemoprevention.

In another study, the effect of turmeric was examined on patients with irritable bowel syndrome. When 1 or 2 tablets of a standardized turmeric extract were given daily for 8 weeks, the prevalence of irritable bowel syndrome was significantlydecreased, as was the abdominal pain/discomfort score (Bundy et al. 2004). Alcoholic extract of turmeric offered protection against BaP-induced increase in micronuclei incirculating lymphocytes of healthy individuals (Hastak et al. 1997). In a subsequent study, the authors treated patients suffering from oral submucous fibrosis (OSF) with turmeric extract (3 g/day) for 3 months. The number of micronuclei from oral exfoliated cells of OSF patients before and after treatment with turmeric extract wasrecorded. They found that the number of micronuclei in oral exfoliated cells decreased substantially and was comparable with that of normal, healthy individuals as in Figure1-1.

Table 1-1: Human Studies with turmeric

Diseases	Dose	Response
Asthma	NC	Relief from bronchial asthma and cough
Cancer	Topical application	Reduction in itching, pain, and size in external cancerous lesions
Mutation	1500 g/day × 30 days	Reduction in urinary excretion of mutagens in smokers
Micronuclei	600 mg/day	Inhibition in micronuclei formation in oral mucosal cells and lymphocytes
Abdominal pain	TRE	Reduction of dumpy and colicky pain
Ulcer	1500 mg	Reduction of peptic ulcer
IBS	144 mg/daily × 8 weeks	Reduction of abdominal pain
Infection	Topical application	Umbilical cord care after cutting
Breathing	500 mg in diet	Activation of hydrogen-producing bacterial flora in the colon, increase in breath hydrogen

IBS = irritable bowel syndrome; TRE = turmeric root extract; NC = not clear.

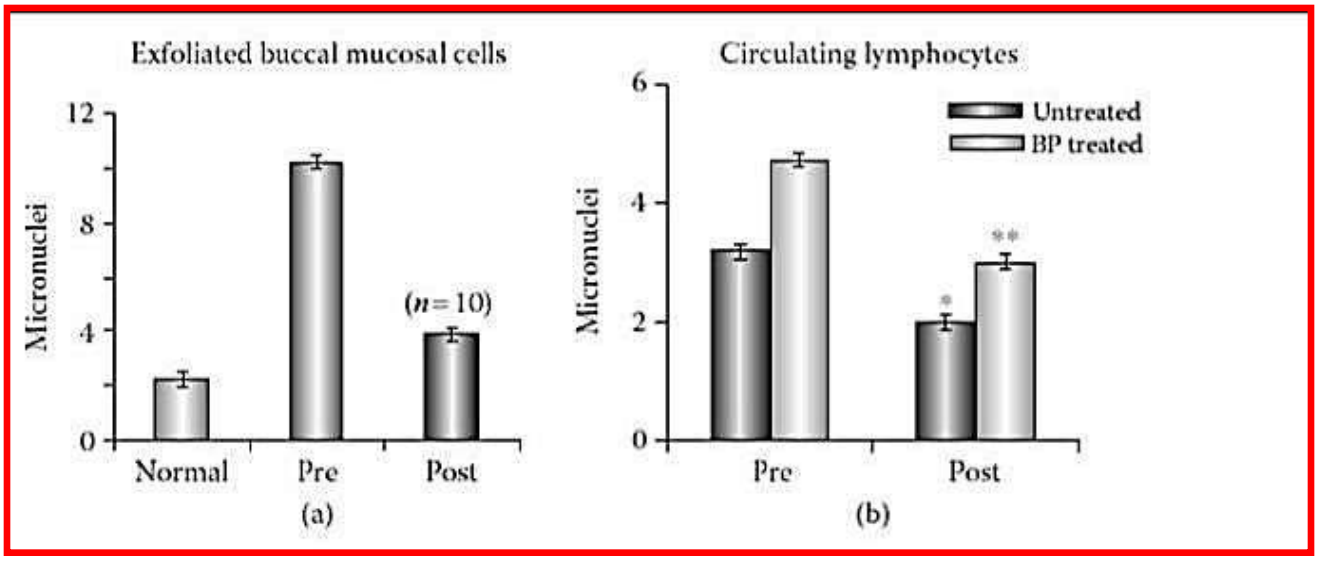

Figure 1-1: the number of micronuclei in oral exfoliated cells decreased substantiallyand was comparable with that of normal, healthy individuals

Inhibition of micronuclei formation in oral submucous fibrosis (OSF) patients: (a) Incidence of micronuclei in exfoliated buccal mucosal cells of OSF patients before andafter treatment with turmeric and of normal healthy individuals. (b) Incidence of micronuclei in circulating lymphocytes of OSF patients before and after turmeric treatment. (n = 10). The symbol * indicates statistical significance when compared with untreated pretreatment group (p < .001). The symbol ** indicates statistical significance when compared with benzo[a]pyrene-treated pretreatment group (p <

.001). (Redrawn from Hastak, K., N. Lubri, S. D. Jakhi et al. 1997. Cancer Lett 116:265–

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Separation of materials

Recent research in separations has been directed towards extending the molecular weight range for these high-resolution methods, while at the same time improving reproducibility and convenience of operation. This overview summarizes some of

these developments and provides insights into the inherent resolution, range, speed, and limitations of these methods.

Important advances in HPLC (Figure 1-2)include the development of better siliceous supports, as well as fresh approaches for producing both covalently-bondedand crosslinked, mechanically-immobilized organic stationary phases that provide a new level of reproducibility and stability for separations. Very wide-pore packings nowpermit separations of species with molecular weights up to about 107 daltons, and columns of particles in the 1–2 μm range allow rapid separations of macromolecules.

Required on a routine basis, and it is also an important industrially applied Separation process for the isolation and purification of natural compounds,

Pharmaceuticals, and other valuable products. However, for achiev-Ing higher purity and recovery yield, high resolution and selectivity are re-Quired. The high resolution in preparative HPLC is highly dependent on Column length. The elongation of columnlength is a simple way to get a Good resolution. However, this will lead to an increasein pressure in the Systems and amount of stationary phase in columns. In addition, changing The length of columns in an industrial place is not available, because of pro-Ducing them in specific dimensions. So, the increase of the columns length Is not economically desirable. The necessity of applying an operating Method that can overcome these limitations while obtaining higher resolu-Tion is attractive. RecyclingHPLC is one way of realizing this. In this technique, the Target compounds return to the column after being in the detector several Times to achieve a higher purification. The benefits of these techniques in-Clude increase of column resolution, higher product purity, and recovery Yield. Also, saving on operating costs may be achieved bynecessity to no Fresh solvents in the recycling periods .

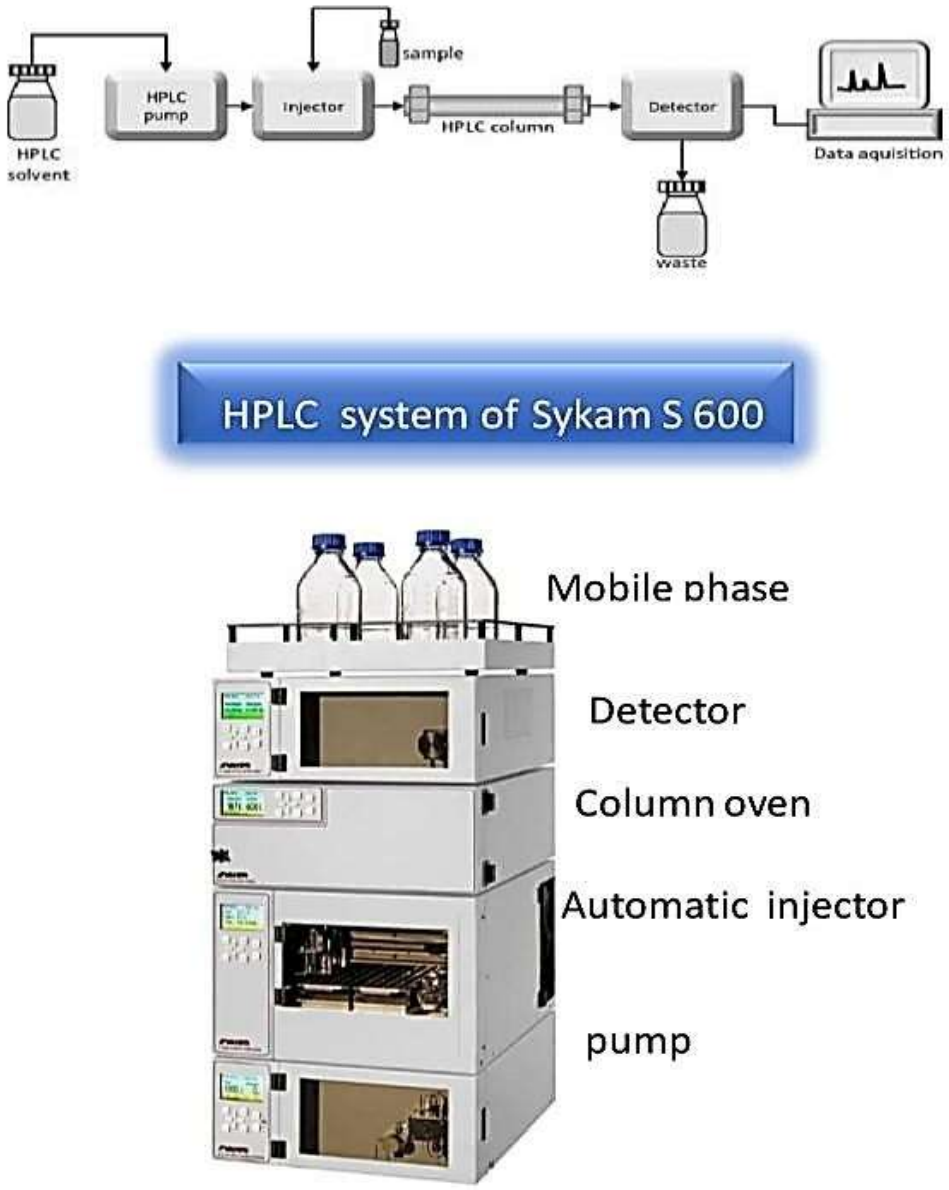

Figure 1-2: Principle and parts of HPLC machine

The result and discussion

Figure 2.1 represents HPLC chromatogram of crude curcumin sample (methanol extracted). There are two significant peaks, the fist is single, high intensity and sharppeak at the retention time 4.4 min. the second is single, sharp but less intensity as a comparison to the first one, the retention time of this peak is 7.2 min. the third low intensity peak at the retention time less than 10 min could be one of the compoundsof the crude curcumin sample or contamination in one of the HPLC parts (injector, column, solvents, ….). This result is indicator for easy separation the two compoundsas there is no overlap between them

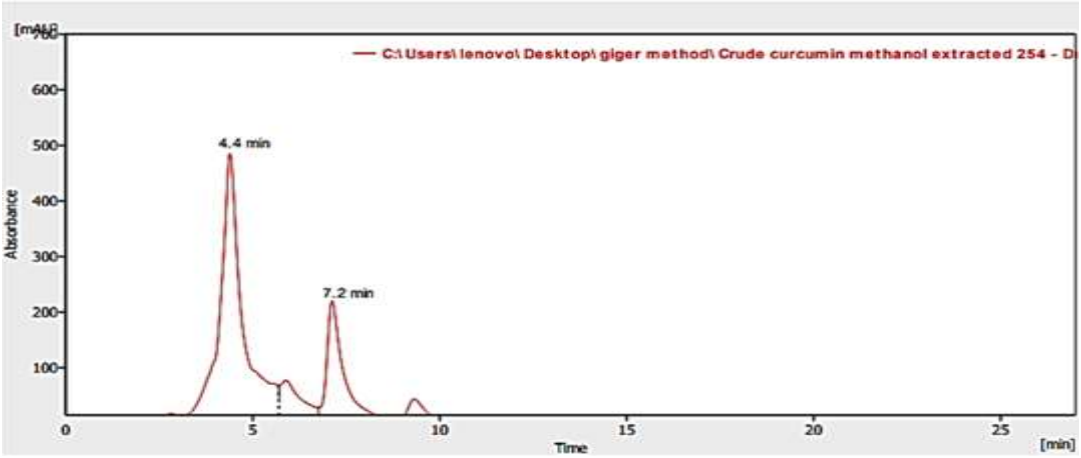

Figure 2-1: HPLC chromatogram of crude curcumin sample (methanol extracted)

Figure 2.2 shows HPLC chromatogram of purified compound at the retention time 4.4 min. The single, sharp and high intensity peak which is consistent with the peak in the figure 2.1 for the crude curcumin sample gives good signal that the used method to separate and purify of this compound was successful. In addition to that, the peak was not broad so this peak represents one compound. Moreover, the retention time of this peak is 4.4 min so it will not be loss of the solvents during collection of the peak as the retention time is not far away.

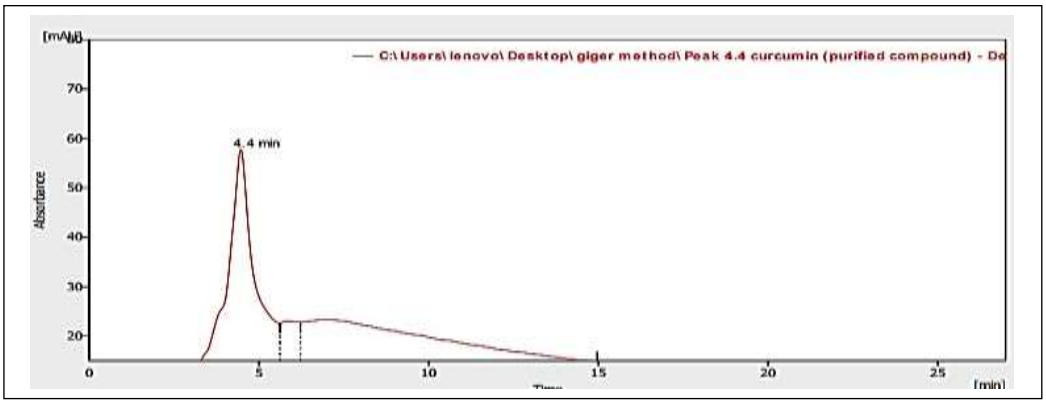

Figure 2-2: HPLC chromatogram of purified compound at theretention time 4.4 min

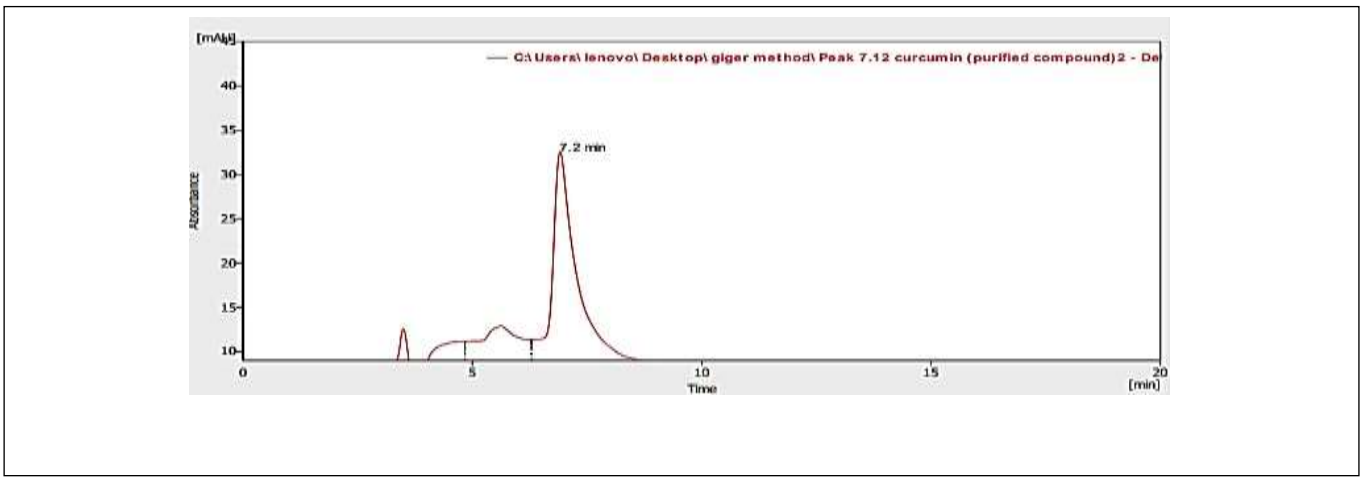

Figure 2.3 clarifies HPLC chromatogram of purified compound at the retention time 7.2 min. the used method to separate of this compound was successful as in thisFigure (2.3), there is single, sharp, high intensity and narrow peak (the starting to ending of the peak is less than one min). according to this result, the process of the collection and purification of this compound is by hand. The other very low intensity peaks at the retention time less than the target peak are could be contaminations orsome of the compounds of the crude curcumin sample were separated but the used method was not suitable for this compounds.

3-Experimental

Analytical methods RP–HPLC

HPLC analysis was performed by a the sykam HPLC S 600,GermanyRP–C18E, 4.6 × 250mm , a 5µL coulum (Germany), injection 10 uL and a flow rate of0.7 ml min–1. Thedetector wavelength was set at 220 nm. The column oven temperature was set at 30
°C. Unless stated otherwise, solvent A was 5% H_2O with 95% acetonitrile and add formic acid 0.1% add to the solvents.

Procedure

1- 250 mg of curcumin powder was grind.

2- 10 mg of curcumin powder was weight and dissolved with methanol.3- The solution was mixed well by the vortex.

4- The sample was sonicated by sonicater for 3 minutes then mixed again by vortex .

5- The sample was centrifuged for 3 minutes then 1 ml of the clear solution wasmoved from eppendrof vial to HPLC vial to be ready for run for HPLC .

6- From result of HPLC 2 purified soluble compounds was collected each alone withcertain volume.

7- The compound was put in the freezer for 24 hour then collected with the freezdryer.

8- 1 mg of the purified compound was weight and dissolved by methanol and checkedby HPLC.

Table 4.1 : It represents a method of separation and purification of compounds

	Gradient Table					
	Time (min.)	**A (%)**	**B (%)**	**C (%)**	**D (%)**	**Flow (ml/min)**
1	Initial	5.0	95.0	0.0	0.0	0.700
2	15.00	20.0	80.0	0.0	0.0	0.700

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