

Characterization of the Purity of Curcumin Extraction: Comparative study of UV Spectrophotometry and High Performance Liquid Chromatography from a Field Application Perspective

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Abstract

Turmeric is a popular spice used in South Asia for both culinary and religious purposes. It has also been used in traditional Indian medicine called Ayurveda. More recently curcumin has been identified to be the active ingredient in turmeric, and its pharmacological basis has been identified. The paper examines the extraction, processing and refinement of curcumin with special emphasis on testing and characterization. This study focuses on the comparison of UV Spectrophotometry and High Performance Liquid Chromatography (HPLC) for characterizing purity of curcumin with the view of selecting the best technique for this particular commercial application. Field application was a key criterion in the selection. Based on the results HPLC was selected as the technique of choice for curcumin characterization application due to various factors such as accuracy of results, low cost, ease of use in the field, simplicity of sample preparation, robustness, quality and reliability.

1.0 Introduction

Turmeric is a popular spice that is commonly used in India and South Asia. It gives the 'yellow-orange' color to curry. In addition to its culinary use, turmeric has been widely used for various purposes in India since time immemorial. In Indian homes, turmeric has been widely used – in cosmetics, as a dye, as a food preservative, as an anti-inflammatory, as a disinfectant, as a depilatory, as a balm to prevent swelling, as a remedy for colds and coughs etc. In Indian temples, turmeric is considered very auspicious to the mother goddess. Finally, related to the topic of this paper – turmeric is used extensively in traditional Indian medicine or Ayurveda.

Ayurveda, the ancient Indian medicine system is now widely recognized today as an effective, alternate and complementary medicine. The word Ayurveda in Sanskrit (an ancient Indian language) can be broken up into two parts - Ayus meaning 'life', and Veda meaning 'science', Ayurveda literally translated is the 'science of life'. Ayurveda entails not only the therapeutic aspects of life, but also takes external parameters into effect such as seasons, climate, plant potency, and daily routine. Historical references of Ayurveda predate around two thousand years ago. The science and practice of Ayurveda was codified around 2,300 years ago by two men: Charaka and Susruta. Their respective treatises – Charaka Samhita and Susruta Samhita dealt with General medicine and surgery respectively. While there have been many subsequent

treatises on Ayurveda, these were the first ones. It is based on these references that modern Ayurvedic doctors perform their duties as physicians.

Plant based medicine is one of the central pillars of Ayurveda. Turmeric has been widely used in Ayurvedic prescriptions as treatment for a wide variety of diseases and conditions, including skin, pulmonary, gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders^[1]. Arthritis is a joint inflammation disorder that affects the lives of millions. Unfortunately, Western Medicine treats arthritis as a condition and cannot 'cure' it, but can only help with its management. Ayurvedic doctors have historically prescribed curcumin to their arthritic patients. Recently there has been a lot of research on the extraction and identification of active ingredients in traditional medicines, specifically to identify their pharmacological basis. Focus of this study was to evaluate typical characterization techniques for testing curcumin purity - UV Spectrophotometry and High Performance Liquid Chromatography (HPLC) with a view of identifying and selecting the best technique from a field lab deployment perspective.

2.0 Pharmacology of Curcumin

Scientists have shown that curcumin exhibits antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal properties – this is line with its use as a disinfectant by the ancients. More recently scientists have studied its anticancer activities as well as its benefits to chronic conditions such as diabetes, allergies, arthritis, and Alzheimer's diseases. Curcumin provides anti-inflammatory effects by preventing the formation of enzymes and cytokines that in turn drive the expression of inflammatory factors. Given the critical role of inflammation in most chronic diseases, curcumin holds a lot of promise for the treatment of neoplastic, neurological, cardiovascular, pulmonary and metabolic diseases. The pharmacodynamics and pharmacokinetics of curcumin have been examined in animals and in humans recently by Aggarwal, et.al^[1,2,6,7]

3.0 Curcumin: The Yellow Extract From Turmeric

What is Turmeric ? Turmeric is the rhizome or the underground stem of the curcuma longa plant, a member of the ginger family, Zingaberaceae. The rhizome which is similar to ginger in that it is rough, is finger like with dark brown scales, is however smaller than ginger. It has a dull orange internal flesh. The rhizome is directly used in certain ayurvedic preparations in the form of a paste. The most common usage involves drying the rhizome and then grinding into dry powder to form the familiar 'curry powder'.



Figure 1: *Curcuma Longa* (Turmeric Plant) with its rhizome or root ^[3]

Turmeric, the curcumin plant, is a tropical plant, grown in South Asian regions. The plant is long, (about a meter in length), and its flowers are white and long. The rhizome of the plant is a maximum of 2 cm long, and a maximum of 7.5 cm in width. The city of Nizamabad in the South Indian state of Andhra Pradesh is popularly called ‘Turmeric City’ because of the abundance of this cash crop. Turmeric is planted with the first monsoon rains, and it takes approximately 8-10 months to mature. [4]

Even though many ancient medical treatments are generally safe, historically they have been ignored by western medical research due to the researchers’ inability to identify their active ingredient(s) as well as its molecular target(s). Turmeric is a notable exception. The key ingredient of turmeric, called curcumin, which gives turmeric its characteristic yellow color, was first isolated over a century ago. That isolation was limited to the identification of the ingredient that gives turmeric its characteristic color in connection with its use as a coloring agent. In the past two decades, extensive research has successfully identified curcumin to be the active ingredient in turmeric that provides its anti-inflammatory properties. Identification of active ingredients was crucial as it paved the way for formulating potent medications that can be distributed through a traditional pharma model.

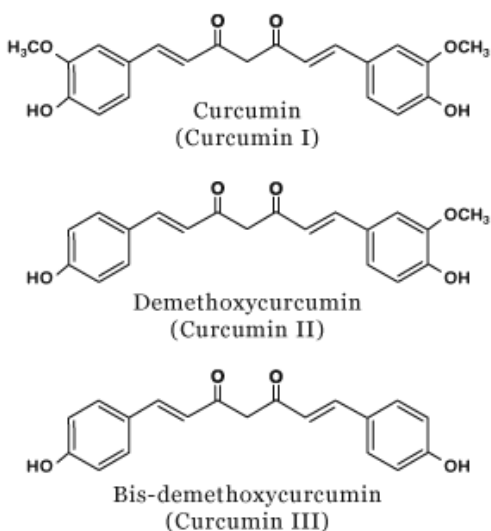
4.0 Extraction and Processing of Curcumin

Turmeric has been found to have the composition as shown in table below ^[4]

Component	%
Curcumin and other curcuminoids	4%
Fiber	4%
Oils	5%
Minerals	5%
Protein	7%
Fat	7.5
Water	9.5%
Carbohydrates	61.5%

Table 1: Typical composition of Turmeric ^[4]

The structures of the curcumin and curcuminoids were determined in 1910 to be diferuloylmethanes^[1, 8], pictured in Figure 2 ^[4]. Curcumin, or curcumin 1, has a molecular formula of $C_{21}H_{20}O_6$. The rhizome of the curcuma longa plant, turmeric, is dried in the sun, then powdered and shipped for common uses. Higher quality turmeric powder is sent to labs for purification and refinement to extract pure curcumin (usually 95% purity), which is what is prescribed by Ayurvedic doctors. Figure 3 shows a photograph of the turmeric rhizome as well as the curcumin that was extracted as part of this study.



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Figure 2: The three structures of curcumin and other curcuminoids ^[4]



Figure 3: Turmeric rhizome and curcumin that was extracted

5.0 Refinement of Curcumin

Curcumin composes only 4% of the turmeric rhizome, therefore necessitating the need for highly precise refinement processes. Typically commercial refinement processes lead to 90-95% purity. In this section the refinement processes used in this study are outlined.

5.1 Filtration

Filtration is the first step in the refinement of curcumin. In this process, turmeric powder is converted into a mixture called the Oleoresin. Ethylene dichloride (EDC or $C_2H_4Cl_2$) was used as a solvent to dissolve impurities. EDC was added to the turmeric powder in the ratio of 3 ml/gm of turmeric powder. 300 ml of ethylene dichloride was added to 100 g of turmeric powder in steady and equal increments and mixed. The resulting mixture was heated to $60^{\circ}C$, and stirred using an automatic stirrer for 1 hour. After an hour, the mixture was cooled, the leftover solvent was decanted, and the extract was filtered into another beaker. The same process was repeated twice, the EDC extracts were collected, and the leftover solvent was decanted away. The filtration process removes impurities from the turmeric. The mixture of EDC and turmeric paste is the oleoresin. 100 g of turmeric powder yielded 25.2 g of Oleoresin. The remaining impurities were filtered out in this process.

5.2 Oleoresin to Curcumin

25.2 g of oleoresin was obtained from the filtration process. Using the same 3 ml of solvent per 1 g compound ratio, 75 ml of Isopropyl alcohol (IPA) was measured in a graduated cylinder. An experimental set-up as shown in Figure 4 was assembled to enable the refinement process. A piece of 200 mesh filter paper was weighed and then put into a funnel and placed

over a Buchner flask that was attached to a vacuum. The 25.2 g of oleoresin was transferred to the filter paper, and placed in the funnel. A small quantity of isopropyl alcohol was added to the funnel. This process was repeated, adding the alcohol in small aliquots, . The filter cloth and the oleoresin on top were taken out of the funnel and weighed to be 6.4 g. The resin was then broken up and using the following dimensional analysis, the approximate volume of isopropyl alcohol needed was calculated:

$$\frac{6.4 \text{ g oleoresin} \times 3 \text{ ml Isopropyl alcohol}}{1 \text{ g oleoresin}} = 19.2 \text{ ml of IPA}$$

The process was repeated again with the 19.2 ml of alcohol. The filter paper/cloth was placed in the flask, and again in small equal amounts the alcohol was poured in. Finally the filter cloth and its contents (pure curcumin) were dried in a hot air oven at 60°C for 1 hour. After an hour, the filter cloth and the contents was weighed to be 5.9705 g (weight of the dry curcumin). The mass of the filter cloth, 1.1311 g, was subtracted from the total mass, to get a mass of contents/ curcumin equal to 4.84 g with significant figures.

It was found that the 25.2 g oleoresin provided only 4.84 g of curcumin. From 100 g of turmeric powder we yielded 4.84 g of pure curcumin, corresponding to 5%. The numbers were in line with typical extraction at Katra Phytochem. Typically, raw turmeric contains 3-10%curcumin, the oleoresin contains 25-30%curcumin, and the curcumin sample that is refined contains 90-98% pure curcumin.

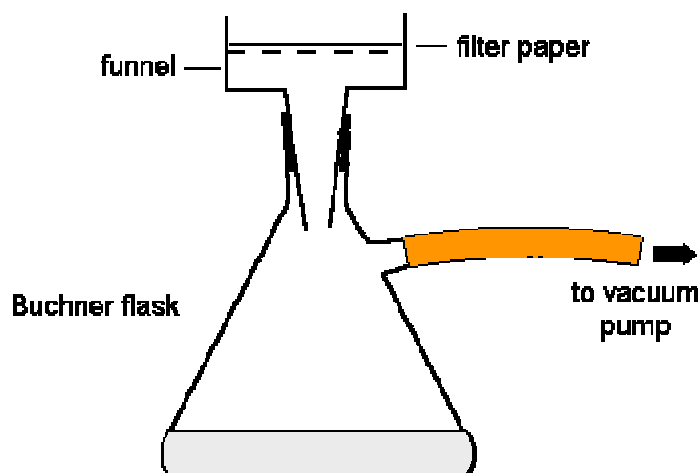


Figure 4. Oleoresin to Curcumin Refinement Process Set-up ^[5]

6.0 Purity Testing Techniques

Purity testing procedures are key as they help to optimize processing procedures as well as to identify the quality of turmeric. Locating characterization labs near the growers enables the growers to optimize turmeric to yield in the field. In this study, two test techniques commonly used for characterizing curcumin were employed, Spectrophotometry and Chromatography. One

of the major goals of this analysis following the research/experiment was to compare these two commonly used techniques specifically for curcumin characterization from a field application perspective. Various factors were identified for this comparison.

6.1 *Spectrophotometry*

Spectrophotometry is an analysis technique that involves the measurement of the amount of light absorbed by a sample material as a function of wavelength. Greater the concentration of the substance in the solution, greater will be the absorption. Thus, by measuring the amount of light absorbed one can calculate the purity of sample through the use of calibrated references.

In this study, a Shimadzu PharmaSpec UV-1700 spectrophotometer with an ultra-violet/visible (UV/Vis) source was used. A good solvent like acetone was used as a reference. Three reference solutions of different ratios of Acetone to water were prepared – baseline (1:1), diluted (1:39) and highly diluted (1:800) for the analysis. A sample of curcumin that needed to be analyzed was weighed to be 0.1078 g. The curcumin sample was added to the sample cuvette along with acetone solution. The two were mixed together using a very small stirring rod. The spectrophotometer was turned on and measurements were obtained as shown in Figure 5.

The following formula was used to calculate percent purity.

$$\% \text{ Purity} = \frac{\text{ABS} \times V_o \times V_{1d} \times V_{2d}}{C \times V_{ac1} \times V_{ac2} \times M_c}$$

Where ABS is the absorption peak from the spectrophotometer data, V_o is the volume of the acetone solution, V_{1d} is the volume of the first diluted acetone solution, V_{2d} is the volume of the highly [second] diluted acetone solution, C is the curcumin factor, 1710, V_{ac1} is the volume of acetone in the first cuvette, V_{ac2} is the volume of the curcumin acetone mixture in the second cuvette, and M_c is the mass of the analyzed curcumin.

Percent Purity Calculation: Results obtained from the spectrophotometer are shown in Figure 5. The Absorption peak was measured to be 0.439 at a wavelength of 419.8 nm.

$$\text{ABS} = 0.439;$$

$$V_o=100 \text{ ml}, V_{1d}=100 \text{ ml}, V_{2d}=100 \text{ ml}, V_{ac1}=5 \text{ ml}, V_{ac2}=5 \text{ ml}$$

$$C=1710; M_c=0.1078 \text{ g}$$

$$\% \text{ Purity} = \frac{0.439 \times 100 \text{ ml} \times 100 \text{ ml} \times 100 \text{ ml}}{1710 \times 5 \text{ ml} \times 5 \text{ ml} \times 0.1078 \text{ g}} = 95.26\%$$

Purity of the curcumin sample was estimated to be 95.26% as measured through spectrophotometry. The procedure was repeated with two more samples. Results are shown in table 2.

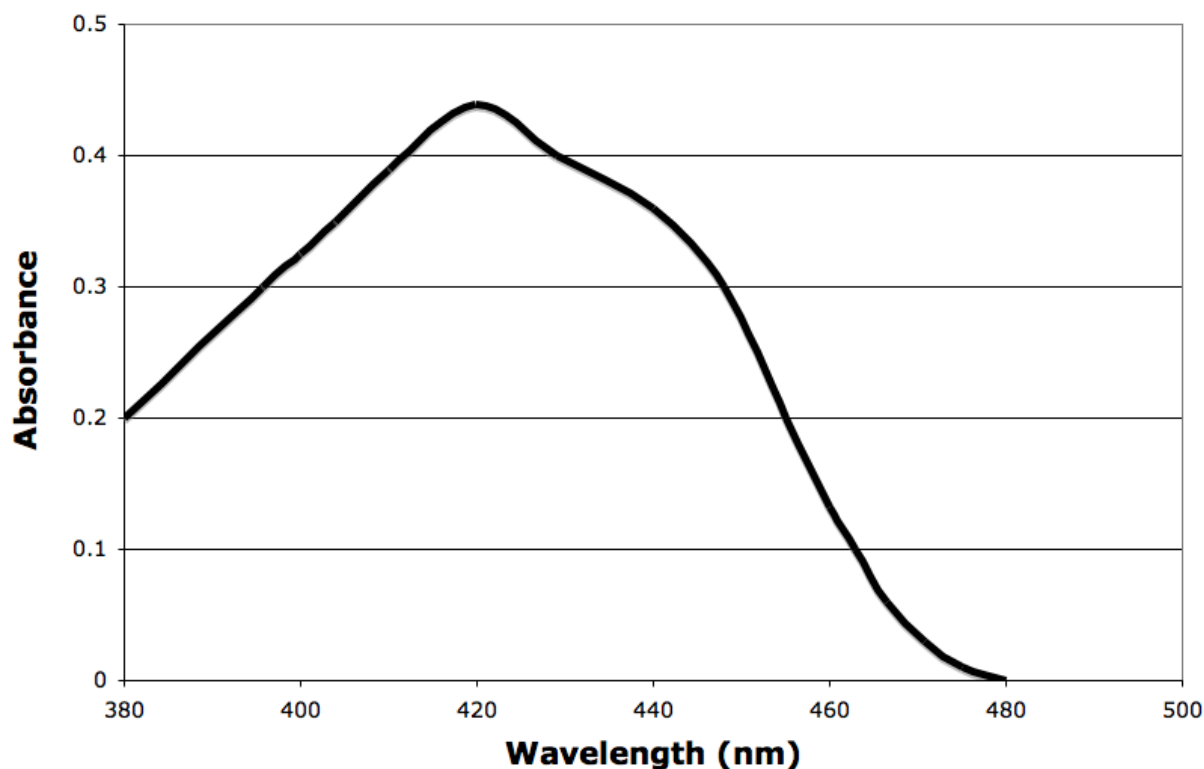


Figure 5: Results from the Spectrophotometer

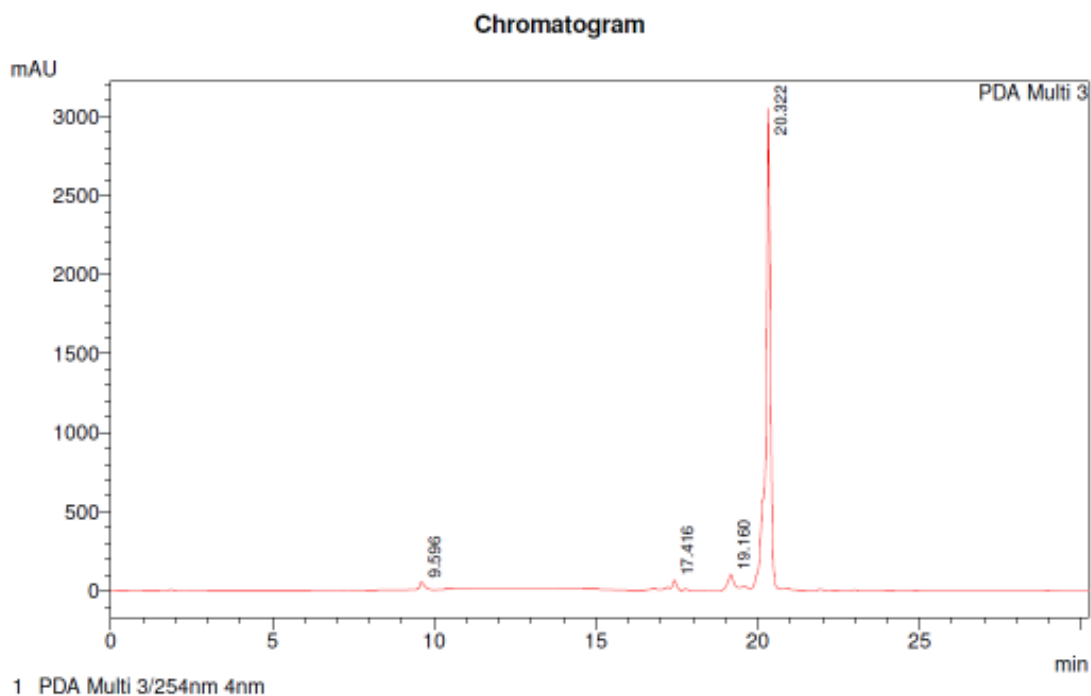
6.2 *Chromatography*

Chromatography is a technique to separate molecules/compounds physically, where the components to be separated are distributed between two phases. There are many types of chromatography: column, liquid, gas, paper, etc. In chromatography, three solvents are used to separate the components of compounds. The solvents can be high polar, mid polar, or non polar. High polar solvents include water, methanol, and ethanol. Mid polar solvents include ethyl acetate, acetone, and chloroform. Non polar solvents include hexane and petroleum ether.

High performance liquid chromatography, or HPLC, was used to test the purity of the curcumin sample. A Shimadzu HPLC machine was used for the analysis.

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E:\SHIMADZU HPLC\Chromatograms\Marker compound\Curcumin crystals - 290711.lcd
 Acquired by : Admin
 Sample Name : Curcumin crystals - 290711
 Sample ID : Curcumin
 Injection Volume : 20 uL
 Data File Name : Curcumin crystals - 290711.lcd



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.596	376217	43735	1.224	1.358
2	17.416	362561	51687	1.179	1.604
3	19.160	928579	86925	3.020	2.698
4	20.322	29078875	3039285	94.577	94.340
Total		30746232	3221633	100.000	100.000

Figure 6: Chromatogram from curcumin Sample

Acetonitrile solution was used as the solvent. A sample of curcumin was weighed to be 2.5 mg and added to the cylinder along with 1000 ml of 60% acetonitrile solution. The HPLC machine was warmed up with a sample of acetonitrile. The acetonitrile was injected into a small hole in the machine to warm-up and clean up the machine. Later, the curcumin/acetonitrile test solution was injected into the machine. The following data was obtained (shown in Figure 6)

Peak number 4 on the data graph is clearly the highest peak that corresponds to curcumin. The area % of this peak was calculated by the instrument to be 94.58% and represents percent purity of the sample. The procedure was repeated with two more samples. Results are shown in table 2.

5.3 Comparison of the two techniques

It was noted that the percentage purities measured using the two techniques differ. For the first sample, results from the spectrophotometer indicated a purity 95.26%, while that from the chromatogram was 94.58%. Results from the three samples are shown in Table 1. The two measurements were quite close to each other and the differences can be attributed to measurement uncertainty. The results have clearly shown that HPLC measurements were in line with the spectrophotometry.

Sample	Spectrophotometry	HPLC
1	95.26	94.58
2	96.53	95.67
3	94.23	93.59

Table 2: Comparison of Results from Spectrophotometry and HPLC

The spectrophotometry technique required fairly sophisticated and expensive instrumentation, elaborate reference solution preparation methods, data interpretation and additional calculations to estimate purity. Spectrophotometry conserved time, but in contrast HPLC was much easier to use. Speed is not a major advantage in this particular application. The sample preparation for HPLC was very simple. HPLC equipment in general was lower cost compared to spectrophotometry. It was also easier to train test personnel on HPLC equipment than spectrophotometry providing greater efficiency. One of the criteria for consideration was field operation – HPLC equipment was deemed to be more suitable for remote field labs for use by technicians with limited qualifications.

Given the results from the two techniques were comparable, this study concluded that the HPLC owing to the reasons stated above was the technique of choice for curcumin characterization from a field application lab perspective.

6.0 Summary and Conclusion

The widespread use of Turmeric was examined, specifically related to medical treatments in traditional Indian medicine. The active ingredient of turmeric was reported along with its pharmacological basis. The chemical extraction was reported on, additionally the processing and refinement of curcumin with special emphasis on testing and characterization of purity was reported. Specifically UV spectrophotometry and High Performance Liquid Chromatography (HPLC) have been compared for the characterization of sample purity. It was shown that while both methods yield comparable results. HPLC was selected to be the technique of choice for the characterization of curcumin owing to its low cost, ease of operation and lack of elaborate sample preparation procedures.

7.0 Acknowledgements

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8.0 References

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