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Extractive Spectrophotometric Methods for the Determination Of A Versatile Nutraceutical: Curcumin

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ABSTRACT: Nutraceuticals are products, which other than nutrition is also used as medicine. A nutraceutical product may be defined as a substance, which has physiological benefit or provides protection against chronic disease. Simple, sensitive, selective, rapid and reliable methods for the spectrophotometric determination of curcumin belonging to the class of neutraceuticals has been worked out. The methods are based on the reaction of curcumin with folinciocalteu (FC), an electrophilic coupling agent in presence of iron (III) in mild hydrochloric acid medium. The bluish –green complex shows maximum absorbance at 650nm. The colour complex can be extracted into chloroform. The methods obey Beer's law. As many as 10 anions and cations do not interfere. The methods have good reproducibility and can be satisfactorily applied to the determination of curcumin in various turmeric samples.

Index Terms- nutraceutical, turmeric, curcumin, curcuma longa, spectrophotometry, folinciocalteu

I.INTRODUCTION

Nutraceutic is a term derived from "nutrition" and "pharmaceutics." The term is applied to products that are isolated from herbal products, dietary supplements (nutrients), specific diets, and processed foods such as cereals, soups, and beverages that other than nutrition are also used as medicine.[1]

In the US, the term "nutraceutical" products are regulated as drugs, food ingredients and dietary supplements. The term is not defined the same in different countries, but is usually defined as a product isolated from foods that is generally sold in medicinal forms not usually associated with food. A nutraceutical product may be defined as a substance, which has physiological benefit or provides protection against chronic diseases.[1] Nutraceuticals may be used to improve health, delay the aging process, prevent chronic diseases, increase life expectancy, or support the structure or function of the body. Nutraceuticals, in contrast to pharmaceuticals, are substances, which usually have not patent protection. Both pharmaceutical and nutraceutical compounds might be used to cure or prevent diseases, but only pharmaceutical compounds have governmental sanction.[2]

Nutraceuticals encompass a large group of preventive and curative health ingredients that have been predominantly derived from long standing medical tradition such as Ayurveda,Tibetian, Chinese and Japanese medical systems.All these systems are primarily dependent upon plants, more commonly known as herbs, especially those with a well –established use as a foodstuff. The blend of these pharmaceuticals and nutritional characteristics resulted in the name "Nutraceuticals" to denote the nutritional origins and the design molded on pharmaceuticals, that is, standardization, efficacy and predictability.

Turmeric is a well-known indigenous herbal medicine, which exhibits a wide range of biological activities, for example, antibacterial [3], anticancer [4], anticoagulant [5], anti-inflammatory [6,7], antimutagenic[7,8], antioxidant[8,9], antiprotozoan[10], antispasmodic[6], antitumour[1112], antiviral[7], hepatoprotective[7], hypocholesterolaemic[13], hypoglycemic[14], hypolipemic[15], besides being effective oxygen species scavengers and lipid peroxidase inhibitor[16].

Turmeric (from the rhizome of Curcuma longa L., family Zingiberaceae) is a well-established foodstuff. It is a well-established foodstuff. It is the main ingredient of curry powders. It is also well-known for its coloring, flavoring and digestive properties. The main yellow pigment (0.5-0.6%) is curcumin [1,7-bis-(4'-hydroxy-3'-methoxy-phenyl)hepta-1,6-diene-3,5-dione]. The minor constituents are suggested to be geometrical isomers of the major constituent [17] collectively called curcuminoids. In brief, the vast success of the herbal medicine during the last two decades has made curcumin a major area in drug

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research laboratories and a branch of commercial importance in nutraceuticals.

The field of nutraceuticals, like modern analytical pharmacy demands separation of a desired component from a complex dosage formulation/ biological materials and its instrumental determination. The potential of curcumin as an important class of nutraceuticals prompted the development of many analytical methodologies for the detection, determination, isolation and characterization.

Analytical techniques including chromatography [18-27] and optical methods [28-32] have been reported. However, these methods have proved to be deficient with respect to specificity, sensitivity, simplicity and or short analysis time. Quantitation of the constituents by TLC [19,24] is ruled out since it hardly leads to consistent results. HPLC is reliable analytical technique for identification of impurities in preformulationn or of metaboloites in biological matrices, rather than for routine quantitative analysis. Further, the cost of instrument is relatively high and maintenance demands sophistication. However, in separate communications [24,25] HPLC is regarded as one of the best analytical technique. For routine nutraceutical applications optical methods such as spectrophotometry seems to be the most attractive analytical approach. It is convenient and simple, and can be relatively inexpensive. Further, this method provides simple, precise and accurate measurement of suitable analytes.

Spectrophotometric estimation of curcuminoids revolves on strong absorption of the compounds in organic solvents between 419 to 430nm. Further, the evaluation of the total amount of curcuminoids in a sample by the use direct absorption measurement is valid, if the calculations are based on 'pure standards'. Besides, the presence of other compounds absorbing in the region 420-430nm will strongly influence the results. Thus, there is a great need to develop a simple, sensitive and rapid spectrophotometric method for the determination of curcuminoids.

The spectrophotometric methods reported for the determination of curcumin may be broadly classified into three groups. Group I methods are based on the direct spectrophometric determination of colour ingredient of curcumin in non-aqueous solvents, such as methanol [31] and DMF [28] while, the group II method utilizes aqueous buffer (pH 11) and the resulting purple colour formed was measured at 520 nm [29]. Group III method is based on the reaction of iron(III) and potassium ferricyanide with curcumin and the absorbance of the resulting complex was measured at 728 nm [28]. All the methods reported are less sensitive.

The work described here is a novel and highly sensitive method for the determination of curcumin which is based on the reaction involving the use of iron (III) salts in the presence of electrophilic coupling reagent folinciocalteu (FC) in mild hydrochloric acid medium. The proposed method offers the advantage of simplicity with respect to reagents, high sensitivity and stability without extraction, heating or distillation and reliability due to reproducibility.

II. EXPERIMENTAL

2.1. Material and methods

Apparatus: UV-VIS spectrophotometer UVIDEC-610 type with 1.0-cm matched cell (Jasco, Tokyo, Japan) was employed for measuring the absorbance values.

2.2. Reagents

Curcumin (Sami lab, India), folinciocalteu (FC) (Sigma, USA) and iron (III) chloride (BDH, India) were used. All other chemicals and solvents were of analytical grade. Double distilled water was used throughout. Curcumin 100 mg) was dissolved in 100-ml of isopropyl alcohol. The stock solution was diluted with isopropyl alcohol to obtain solutions of required concentrations. Aqueous solutions of folinciocalteu (0.1% w/v), iron (III) chloride (0.5% w/v) containing few drops of 2N (v/v) hydrochloric acid was prepared. Solution of folinciocalteuwas stored in amber bottle to protect from sunlight and ethyl alcohol was distilled before use. Chloroform (Ranbaxy, India) was used as received. Solutions of anions and cations were prepared by dissolving their corresponding salts.

2.3. Procedures

(i)Direct spectrophotometry(MethodA)

Aliquots of standard solutions of curcumin were transferred into 10-ml calibrated flasks. 1.0 ml of folinciocalteu (0.1% w/v) and 2.0 ml of iron (III) chloride (0.5% w/v) was added and after 10 min the solutions were made upto mark using alcohol. The absorbance was measured at 650 nm against the corresponding reagent blank and calibration graphs was constructed.

(ii)Extractive spectrophotometry (Method B)

Appropriate volume of standard curcumin solution, 1.0 ml of folinciocalteu (0.1% w/v) and

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2.0 ml of iron (III) chloride (0.5% w/v) and 2.0 ml of ethyl alcohol were added to a 125-ml separating funnel. To this 6.0 ml of chloroform was added and the contents were extracted. The organic layer was collected and passed over about 1.0 g of sodium sulphate and made upto mark using chloroform in 10-ml calibrated flask. The absorbance was measured at 650 nm against the corresponding reagent blank and calibration graphs was constructed.The optical characteristics determined are presented in Table 1.

III. RESULTS AND DISCUSSION

Curcuminoids are the major active constituents of turmeric. The determination of curcuminoids in turmeric is crucial to determine the quality of plant material or its processed products.

Folin-ciocalteu(FC) is an electrophilic coupling reagent and is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric in vitro assay of phenolic and polyphenolic antioxidants.[33]. This reagent does not measure only phenols, but will react with any reducing substance. It therefore measures the total reducing capacity of a sample, not just phenolic compounds. This reagent is part of the Lowry protein assay, and will also react with some nitrogen-containing compounds suchas hydroxylamine and guanidine[34].The reagent has also been shown to be reactive towards thiols, many vitamins, the nucleotide base guanine, the trioses glyceraldehyde and dihydroxyacetone, and some inorganic ions. Copper complexation increases the reactivity of phenols towards this reagent.[35].

Table 1: Spectral data for the determination of curcumin using Folinciocalteu

Parameters	DS(MethodA)	ES (Method B)
Colour	Bluish green	Bluish green
λ_{max} (nm)	650	650
Stability (h)	8	8
Beer's law (ng ml^{-1})	0.2-7.0	0.1-6.0
Recommended drug concentration (ng ml^{-1})	3.0	2.0
Molar absorptivity (L mol^{-1} cm^{-1})	5.96×10^4	6.89×10^4
Sandell's sensitivity (µg cm^{-2})	0.007	0.006
Regression equation*		
Slope (a)	0.0645	0.1061
Intercept (b)	0.0664	-0.0087
Correlation coefficient	0.9876	0.9943

*y=ax+b where x is the concentration of curcumin in µg ml^{-1}

3.1. Reaction mechanism

The chemical reaction in the procedure described for the spectrophotometric determination of curcumin involves the reduction of iron(III) chloride by FOLIN CIOCALTEU which subsequently couples with curcumin to form a bluish-green product having a maximum absorption at 650 nm. The colour intensity remains constant for 8 h. Addition of a few drops of 2N HCl(v/v) is necessary to prevent precipitation of iron (III) as hydrated ferric oxide. The factors affecting the colour development, reproducibility, sensitivity and adherence to Beer's law were investigated.

3.2. Spectral characteristics

A bluish-green coloured product with maximum absorbance at 650nm was formed when curcumin was allowed to react with iron(III) chloride in the presence of folinciocalteuin mild hydrochloric acid medium.

3.3. Optimization of analytical variables

For a fixed concentration of curcumin and folinciocalteu the colour intensity remains constant with 1.5-4.0 ml of (0.5% w/v) of iron (III) chloride. Hence, 2.0 ml of iron (III) chloride was sufficient for routine analysis. Similar procedures were adopted to know the amount of folinciocalteu required for constant colour intensity. It was found that 0.5-4.0 ml of folinciocalteu (0.1% w/v) was required to provide maximum colour intensity and stability. Hence, 1.0 ml of 0.1% (w/v) of folinciocalteu was found to be optimum to get reproducible results.

3.4. Order of addition

The sequence of addition of curcumin,folinciocalteu and iron (III) chloride was studied via the formation of the bluish-green complex. The study indicated that the sequence of addition of reactants had profound influence on the

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intensity and the stability of the colour, for example, (1) folinciocalteu+iron(III)chloride +curcumin and (2) iron (III) chloride+ curcumin+folinciocalteu gave less intense and unstable colour. While, the order: (3) curcumin +folinciocalteu+iron (III) chloride gave more intense stable bluish-green colour.

3.5. Temperature and stability

Development of bluish-green colour was carried out at room temperature and this intensity decreases rapidly when diluted with water or when the temperature is increased. Ethyl alcohol stabilizes the colour for more than 8 h. Isopropyl alcohol was the preferred solvent for preparing stock solution of curcumin as ethyl alcohol and methyl alcohol interfered only, if added before the development of the colour. Subsequently, both the solvents do not interfere in the reaction. Conversely, isopropyl alcohol is discouraged, as it is costlier to ethyl alcohol and methyl alcohol. Ethyl alcohol was preferred to methyl alcohol as it is nontoxic.

Acids like hydrochloric, sulphuric, nitric and perchloric; solvents like acetone, acetic acid, acetonitrile were not effective in stabilizing the colour; while bases such as sodium hydroxide and ammonia were found to give a red colour with curcumin. Conversely, methyl alcohol and ethyl alcohol have profound influence and enhances the stability of the colour and forroutine analysis, ethyl alcohol is preferred as it is nontoxic and is cost effective.

3.6. Calibration and spectral data

The bluish-green colour obeyed Beer'law. The optical characteristics such as optimum range, as evaluated from a ringbom plot, molar absorptivity, sandell'ssensititity, slope, intercept, correlation coefficient are shown in Table 1.

3.7. Interference

The effect of various anions and cations on the determination of curcumin was studied as per the proposed procedure and the results are presented in Table 2 and Table 3. In general, 100 mg of the salt was added individually to aliquots containing 4.0 μg $ml^{-1}$and 3.0 μg $ml^{-1}$of curcumin with iron(III) and folinciocalteu for method A and B, respectively.

Table 2: Effect of anion on the determination of curcumin

Salt of the anion added	Salt added mg	% Recovery of curcumin*±RSD**	
		Method A	Method B
Ammonium tartarate	100	99.8 ± 0.53	99.4 ± 1.03
Calcium carbonate	100	98.2 ± 1.02	98.2 ± 0.52
Potassium bromate	100	100.6 ± 1.09	99.6 ± 0.76
Potassium chloride	100	99.6 ± 0.98	98.8 ± 0.82
Potassium iodate	100	97.8 ± 0.66	100.4 ± 0.96
Potassium sulphate	100	98.4 ± 0.68	98.8 ± 0.96
Sodium fluoride	100	98.6 ± 1.04	99.6 ± 0.80
Sodium nitrate	100	99.4 ± 0.74	100.4 ± 0.78
Sodium phosphate	100	99.4 ± 0.62	98.6 ± 0.86
Sodium sulphate	100	99.6 ± 0.84	99.6 ± 1.06

*3.0 μg $ml^{-1}$and 2.0 μg $ml^{-1}$of curcumin for method A and B, respectively
** relative standard deviation(n=5)

Table 3: Effect of cation on the determination of curcumin

Salt of the anion added	Salt added mg	% Recovery of curcumin* ± RSD**	
		Method A	Method B
Ammonium molybdate	100	99.8 ± 0.70	98.4 ± 0.54
Barium sulphate	100	96.8 ± 0.80	99.4 ± 0.65
Cadmium sulphate	100	99.4 ± 0.52	100.6 ± 0.98
Lead nitrate	100	98.6 ± 1.07	98.7± 0.86
Magnesium sulphate	100	97.5 ± 0.66	99.4 ± 0.79

Manganese sulphate	100	96.4 ± 0.54	98.2 ± 0.65
Potassium chromate	100	98.2 ± 0.65	99.5 ± 0.87
Strontium nitrate	100	98.7 ± 1.02	98.4 ± 0.67
Tin chloride	100	99.8 ± 0.98	99.6 ± 0.79
Zinc sulphate	100	99.3 ± 0.76	98.6 ± 0.86

*3.0 µg ml^{-1} and 2.0 µg ml^{-1} of curcumin for method A and B, respectively
** relative standard deviation(n=5)

3.8. Applications

Powdered turmeric (0.1g) was mixed with 10.0 ml of light petroleum (boiling range 40-60 °C) and allowed to stand overnight. The solvent was discarded and the powder was extracted with 10.0 ml of isopropyl alcohol. An aliquot of this solution was analyzed by the proposed method. Known amount of curcumin was added to the same solution and recovery experiments were performed. The results are presented in Table 4.

Table 4: Determination of curcumin in various turmeric samples

Sample	Amount of curcumin found by proposed method %	Amount of curcumin added to the same sample solution (g)	Amount of curcumin found by difference (g)	Recovery%
Wynad*	3.5	0.1	0.963	96.3
Alleppey*	3.7	0.1	0.998	99.8
Salem*	3.1	0.1	0.973	97.3
Rajapuri*	2.8	0.1	0.962	96.2
Warangal*	3.0	0.1	0.993	99.3

*name of the place in India where turmeric is grown

IV CONCLUSION

The herbal renaissance has produced a profound effect on the western medicine, which is now trying to acknowledge methods of healing that have existed for millennia in the traditional medicine throughout the world, especially Asia. The surge in research on drugs from natural sources is now moving out of the herbalists shop away from the core texts into the drugs research laboratories. With increasing consumer awareness, the pharmaceutical industries in drug control authority have long been interested in the development of simple and sensitive methods for the assay and evaluation of drugs in bulk and in dosage forms, to assure high standard of quality control. In the present context, determination or estimation of nutraceuticals in plant material or processed products are of paramount importance. Simple methods based on spectrophotometry may dominate as analytical tool for the evaluation of nutraceuticals. Our methods are a step towards this direction.

The proposed spectrophotometric methods have adequate sensitivity and accuracy for determination of curcumin in various turmeric samples. Their analytical characteristics such as sensitivity, selectivity and stability excelled other existing spectrophotometric methods. Although, HPLC is presently one of the most common and powerful techniques for curcumin determination, the results show that the proposed methods can provide almost similar results with considerably low cost. Visible spectrophotometer is easily available in most laboratories for routine determination of curcumin, especially in developing countries.

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