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SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF A COSMECEUTICAL: TETRAHYDROCURCUMIN

Syeda Ayesha*

*Department of Chemistry, Government First Grade College, Kuvempunagar, Mysore 570023, India

***Corresponding Author:** Syeda Ayesha
*Email: ayeshasyeda2005@gmail.com

Abstract
Tetrahydrocurcumin (THC), a cosmeceutical is one of the major metabolite and reduced form of curcumin. Simple, sensitive, selective, rapid and reliable method for the spectrophotometric determination of tetraydrocurcumin has been developed. The method describes the electrophilic coupling reaction of tetrahydrocurcumin with 3-methyl-2-benzothiazoline hydrazono hydrochloride hydrate (MBTH) in presence of iron (III) or cerium(1V) to produce a pink product having maximum absorbance at 530 nm in mild sulphuric acid media. The pink colour complex can also be extracted into chloroform. The methods obey Beer's law. As many as ten each of anions and cations listed do not interfere.

Keywords: Tetrahydrocurcumin; MBTH; Cosmeceutical; Nutraceutical

1.Introduction

The resurgence for alternate medicine globally supported by safe and cost effective and more accessible health products has resulted the emergence of new field of activities of nutraceuticals and cosmeceuticals. Nutraceuticals encompass a large group of preventive and curative health ingredients derived from herbs, especially those with a well-established use as foodstuff and the design molded on pharmaceuticals. Cosmeceuticals are nutraceuticals, which display cosmetic properties.

Tetrahydrocurcumin is the metabolite of curcumin. Synthetically, it is obtained by the reduction of curcumin [1]. Tetahydrocurcumin, the hydrogenated derivative of curcumin displays the same beneficial activities as shown by curcumin [2]. It has the same reactive sites as that of curcumin *viz*. two ortho-methoxy phenolic groups and a reactive methylene group [3].

Tetrahydrocurcumin is a beta-diketone in which both of the double bonds have been reduced to single bonds. Tetrahydrocurcumin is a derivative of curcumin, a natural compound found in turmeric. While curcumin has been extensively studied for its health benefits, tetrahydrocurcumin is a more potent and bioavailable form of the compound.

Tetrahydrocurcumin, a cosmeceutical is colourless reduced form of yellow curcuminoids extracted from the roots of Curcuma longa, commonly called turmeric root [4]. Tetrahydrocurcumin exhibits strongest antioxidant activity among curcuminoids studied in several vitro systems [5,6]. They may therefore be used in colour - free foods and cosmetic products, which currently employ conventional synthetic antioxidants such as butylated hydroxytoluene. An antioxidant used in a cosmetic application should have the capability of efficiently quenching any radicals on the surface of the skin. Tetrahydrocurcumin displays free radical scavenging ability and skin lightening actions [6]. It also displays anti-inflammatory [6] and anti-cancer activity [7]. Protective role of THC against erythromycin estolate induced hepatotoxicity has also been reported [8]. The structural studies of THC have been reported [9]. The molecule is non-planar and the benzene rings positioned at the ends of heptane chain are orthogonally placed, with a dihedral angle of 84.09(7) ° between them. The study was carried out to confirm the reports that the p-hydroxy functional groups are responsible for the antioxidant and chemo preventive action of the compound [10].

This paper is an attempt to meet an ever-increasing demand for the analytical control of commercialized health care products by developing simple, sensitive, selective, rapid and reliable spectrophotometric procedures for the determination of newly introduced cosmeceutical product. Survey of the literature revealed that no analytical method has been reported for the determination of the cosmeceutical. We report first ever spectrophotometric methods for the determination of THC in pure and laboratory prepared formulation. The latter type of reaction
involves the oxidative coupling of THC with 3-methyl-2-benzothiazoline hydrazono hydrochloride hydrate (MBTH), an electrophilic coupling reagent, in the presence of an oxidant iron (III) or cerium (IV) in mild sulphuric acid medium and the measurement of the resulting pink chromophore. The proposed methods have distinct advantages of sensitivity and stability and offers flexibility for direct and extractive spectrophotometry. Also, the method does not require heating or distillation and exhibits reliability due to reproducibility.

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2.Experimental

2.1. Apparatus

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

2.2. Reagents

Tetrahydrocureumin (THC) (Sami lab, India), a methyl 2- benzothiazoline hydrazono hydrochloride (MBTH) (Sigma, USA), iron (III) chloride, cerium (IV) sulphate (BDH, India), chloroform, isopropyl alcohol (Ranbaxy, India) was used as received. Alcohol was distilled before use and double distilled water was used throughout. Tetrahydrocurcumin (100mg) was dissolved in isopropyl alcohol in a 100-ml volumetric flask and made up to mark. The solution was further diluted with distilled water to get solutions of required strength. Aqueous solution of MBTH (0.05% w/v), iron (III) chloride (0.1% w/v) containing few drops 2N (v/v) hydrochloric acid and cerium (IV) sulphate (0.1% w/v) containing 5.0 ml of IN sulphuric acid was prepared. Solution of MBTH was stored in amber bottle to protect from sunlight.

2.3. Procedures

2.3.1. Direct spectrophotometry

Aliquots of standard solutions of tetrahydrocurcumin, 1.0 ml of MBTH and 1.0 ml of iron (Ill) chloride or cerium (IV) sulphate were transferred in a series of 10-ml calibrated flasks. The contents were mixed well and allowed to stand for 10 min. It was then made up to 10-ml mark using alcohol and the flasks were again kept for 15 min. The absorbance was measured at 530 nm against the corresponding reagent blank and calibration graphs were constructed.

2.3.2. Extractive Spectrophotometry

Standard solution of tetrahydrocurcumin, 1.0 ml of MBTH and 10 ml of iron (III) chloride or cerium (IV) sulphate was taken in a 125-ml separating funnel. After 10 min, 3.0 ml of alcohol was added and the contents after mixing well were kept as such for another 5 min. The solution was extracted with 6.0 ml of chloroform, the organic layer collected was passed over about 1.0 g of anhydrous sodium sulphate and the volume was made up to 10-ml using chloroform. The absorbance was measured at 530 nm against chloroform. Calibration graphs were constructed and the optical characteristics for the determination of tetrahydrocurcumin are presented in Table I.

3. Results and Discussion

MBTH is an electrophilic coupling reagent. It was first introduced for the determination of aromatic amines, imino heteroatomic compounds and aliphatic aldehydes. Later, it was extended for the determination of a large number of organic compounds such as those containing groups, carbonyl compounds, schiff's bases, aromatic hydrocarbons, saccharides, steroids, olefins, phenols, furfural and heterocyclic bases [11].

3.1. Reaction mechanism

The chemical reactions in this method involves the reduction of iron (III) chloride or cerium (IV) sulphate by MBTH which subsequently couples with the tetrahydrocurcumin to form a pink product having maximum absorption at 530 nm. The procedure described for the spectrophotometric determination of tetrahydrocurcumin with MBTH in presence of cerium (IV) sulphate are often complicated by a number of problems which are not always apparent from the literature. For example, MBTH performs well in alkaline medium [8] but, in the present case, precipitation which increases with increase in pH of the solution has been observed. To overcome this problem the authors performed the reaction in acidic medium, which is essential in the present case as optimum acidity is required for the coupling reaction, because ceric hydroxide will start to precipitate at values above pH 1. The factors affecting the colour development, reproducibility, sensitivity and adherence to Beer's law were investigated.

3.2. Spectral characteristics

A pink product with maximum absorption at 530 mm was formed when tetrahydrocurcumin was allowed to react with iron (III) chloride or cerium (IV) sulphate in mild sulphuric acid medium in the presence of MBTH.

3.3. Optimization of analytical variables

For a fixed concentration of tetrahydrocurcumin and MBTH, the colour intensity remains constant with 0.5-2.0 ml of iron (III) chloride (0.1%w/v) or 0.70-2.0 ml of cerium (IV) sulphate (0.1% w/v) solution. Hence, 1.0 ml of iron III) chloride or cerium (IV) sulphate was sufficient for routine analysis.

Similar procedures were adopted to know the amount of MBTH required for constant colour intensity. It was found that 0.5-2.0 ml of MTH (0.05%w/v) was required to provide maximum colour intensity and stability, so 1.0 ml of MBTH was found to be optimum to get reproducible results.

The sequence of addition of tetrahydrocurcumin, MBTH and iron (III) chloride or cerium (IV) sulphate was studied via the formation of the pink complex. The study indicated that the sequence of addition of reactants had profound influence on the intensity and stability of the colour, for example; (l) MBTH + iron (III) chloride or cerium (IV) suphate + tetrahydrocurcumin and (2) iron (III) chloride or cerium (IV) sulphate + tetrahydrocurcumin + MBTH gave less intensive

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and unstable colour. While the order (3) tetrahydrocurcumin +MBTH + iron (III) chloride or cerium (V) sulphate gave more intense and stable colour.

3.4. Effect of solvents

Development of the coloured products was caried out at room temperature. The coloured products were stable for 6 h. For direct spectrophotometry, the colour intensity decreases rapidly when diluted with water, hence the pink colour product was diluted to mark with ethyl alcohol. Isopropyl alcohol was the preferred solvent for preparing stock solution of tetrahydrocurcumin as ethyl alcohol and methyl alcohol interfered in the development of colour. 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 can be used for dilution purposes. however, the use of isopropyl alcohol is discouraged, as it is more costly let alcohol and methyl alcohol. Ethyl alcohol was preferred to methyl alcohol as it is nontoxic.

3.5. Calibration and spectral data

The pink colour complexes obeyed Beer's law. The optical characteristics, such as optimum range, as evaluated from a Ringbom plot, molar absorptivity, sandell's sensitivity, slope, intercept, correlation coefficient is shown in Table 1.

Table 1: Spectral data for the determination of tetrahydrocurcumin

Parameters	Iron (III)		Cerium (IV)	
	DS	ES	DS	ES
Colour	Pink	Pink	Pink	Pink
λ_{max} (nm)	530	530	530	530
Stability (h)	6	6	6	6
Beer's law (ng ml^{-1})	0.5-13	0.25-12	0.5-13	0.2-12
Recommended drug concentration (µg ml^{-1})	5.0	4.0	5.0	4.0
Molar absorptivity (L mol^{-1} cm^{-1}) $x10^4$	2.31	2.75	2.36	2.86
Sandell's sensitivity (µg cm^{-2})	0.012	0.016	0.013	0.015
Regression equation*				
Slope (a)	0.0589	0.0650	0.0512	0.1678
Intercept (b)	0.0140	0.0374	-0.0021	-0.076
Correlation coefficient	0.9992	0.9985	0.9876	0.9897
R.S.D. %**	±0.92	±1.14	±0.97	±1.12

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

** relative standard deviation(n=5)

3.6. Interference

The effect of various anions and cations on the determination of tetrahydrocurcumin was studied as per the proposed procedures and the results are presented in Table 2 and 3. In general, 100 mg of the salt was added individually to aliquots containing 5.0 ug ml of tetrahydrocurcumin .

Table 2: Effect of anion on the determination of tetrahydrocurcumin

Salt of the anion added	Salt added mg	% Recovery of tetrahydrocurcumin* ± RSD**	
		Iron (III)	Cerium (IV)
Ammonium phosphate	100	99.7 ± 0.97	98.4 ± 0.53
Calcium carbonate	100	99.3 ± 1.08	98.9 ± 0.93
Potassium bromate	100	101.0 ± 0.65	99.6 ± 0.82
Potassium chloride	100	99.8 ± 0.43	100.3 ± 0.86
Potassium iodate	100	99.5 ± 0.99	99.7 ± 0.94
Potassium sulphate b	100	99.9 ± 0.76	99.4 ± 0.72
Sodium fluoride	100	100.9 ± 0.76	98.5 ± 1.02
Sodium nitrate	100	98.8 ± 090	100.7 ± 0.53
Sodium phosphate	100	100.9 ± 0.43	99.8 ± 0.71
Sodium sulphate	100	99.4 ± 0.57	100.3 ± 0.98

*5.0 µg ml^{-1} of tetrahydrocurcumin

** relative standard deviation(n=5)

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Table 3: Effect of cation on the determination of tetrahydrocurcumin

Salt of the cation added	Salt added mg	% Recovery of tetrahydrocurcumin* ± RSD**	
		Iron (III)	Cerium (IV)
Copper sulphate	100	100.8 ± 0.61	100.7 ± 0.73
Barium sulphate	100	99.5 ± 0.76	99.1 ± 1.02
Cadmium sulphate	100	100.7 ± 0.73	100.2 ± 0.88
Lead nitrate	100	99.1 ± 1.02	99.7 ± 0.94
Magnesium sulphate	100	100.2 ± 0.88	98.9 ± 0.93
Manganese sulphate	100	99.7 ± 0.94	99.6 ± 0.82
Potassium chromate	100	99.4 ± 0.72	100.3 ± 0.86
Strontium nitrate	100	98.5 ± 1.02	99.5 ± 0.67
Tin chloride	100	100.7 ± 0.53	99.0 ± 1.01
Zinc sulphate	100	99.8 ± 0.92	98.4 ± 0.53

*5.0 µg ml^{-1} of tetrahydrocurcumin
** relative standard deviation(n=5)

4.Conclusion

With increasing consumer awareness, the health care department have been interested in development of simple and sensitive methods for the assay and evaluation of health care products in bulk and dosage forms to assure high standard of quality control. The present trend is in the direction of improvement of physico-chemical methods of analysis. It is envisaged that simple methods based on spectrophotometry will become an accepted analytical tool for the assay and evaluation of health care products. The procedures described in this paper meets most of the demands of analytical chemists namely selectivity, sensitivity, simplicity, rapidity, and cost of analysis.

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