JOURNAL OF CRITICAL REVIEWS

ISSN-2394-5125 VOL 03, ISSUE 02, 2016

DETERMINATION OF CURCUMIN BASED ON COUPLING WITH A CLASS OF SULFA DRUGS

Syeda Ayesha[1*]

[1*]Government First Grade College, Kuvempunagar, Mysore-570023, India. Email: ayeshasyeda2005@gmail.com

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

Abstract:
Sulfanilamide (SAA), sulfadoxine (SDX) and sulfamethoxazole (SMX), the widely used sulfa drugs are proposed as new coupling agents for the spectrophotometric determination of curcumin. The methods are based on the interaction of diazotized sulfa drugs with curcumin to produce a yellow-coloured product with a maximum absorption at 425 nm. The colour developed was stable up to 24h. The methods obey Beer's law. The methods can be successfully employed for the determination of curcumin in presence of common excipients like glucose, lactose, dextrose, starch, sodium alginate and sodium lauryl sulphate, which do not interfere in the proposed methods.

Key words: curcumin; diazotization; sulfanilamide; sulfadoxine; sulfamethoxazole; spectrophotometry

Introduction

Food products, which other than nutrition used as medicines are considered as nutraceuticals. A nutraceutical product is therefore a substance which cures or prevents chronic problems and also provides physiological balance. The words "nutrition" and "pharmaceutics" together constitute the term nutraceutic. Nutraceuticals are substances that are obtained from plants, cereals, specified diet. They are also in fact the substances which in addition to nutrition are also used as medicine [1].

In this era of marketing world, nutraceuticals are used to imply a pharmaceutical effect from a compound or food product that has not been scientifically confirmed or approved to have clinical benefits, but in the global market, there are issues which are related to product quality [2,3]. The use of organic and exotic ingredients in nutraceuticals in the international market lacks regulation which may compromise the safety and effectiveness of products. Competition and survival in the industry has led the manufacturing of unregulated products with low-quality or ineffective ingredients.

Turmeric is a mixture of dried and fresh rhizomes of the plant curcuma longa which belongs to family Zingiberaceae. Traditionally, the plant Curcuma longa is widely used to impart flavor and colour to the food. Curcumin is the active ingredient of turmeric; turmeric contains 2.85% to 6.14% *w/w* Curcumin. Curcumin is freely soluble in methanol, chloroform, ethanol and acetone but practically insoluble in water. Curcumin has the ability to suppress both acute and chronic inflammation and also helps to prevent the damage of the skin from UV rays of the sun [4, 5]. Turmeric is an active ingredient and used in several biological activities, for example, antibacterial [6], anticancer [7], anticoagulant [8], anti-inflammatory [9,10], antimutagenic [10,11], antioxidant [11,12], antiprotozoan [13], antispasmodic [9], antitumor [14,15], antiviral [10], hepatoprotective [10], hypocholesterolemia [16], hypoglycemic [17], hypolipemic [18], besides being effective oxygen species scavengers and lipid peroxidase inhibitor [19].

Curcumin is a major isolated polyphenol from the rhizome of turmeric (*Curcuma longa*). It has a wide range of pharmacological effects such as antioxidant, anti-inflammatory, antimicrobial, antitumor, and hepatoprotective activities. It is the main yellow pigment [1,7-bis-(4'-hydroxy-3'-methoxy-phenyl) hepta-1,6-diene-3,5-dione]. It is already reported that it consists of the minor and major components which are geometrical isomers [20]. In short, the importance of the herbal derived products during the recent times have made curcumin an important field in research techniques.

Many analytical methods *viz* chromatography [21-30] and optical methods [31-35] for the determination of curcumin are reported. But the previous methods lack specificity, sensitivity, simplicity and or short analysis time. Curcumin was also analyzed by TLC [22,27] methods which provided the quantitation of the constituents but, this method is ruled out as inconsistent results were obtained. HPLC [27,28] is reliable method but the cost of the instrument is relatively higher when compared to spectrophotometric methods. But for routine nutraceutical determination spectrophotometric methods are the most precise and accurate. Considering all these lacunae these methods for the spectrophotometric determination of curcumin was of great need.

Curcumin determination by optical methods such as spectrophotometry consists of three types. The first type consists of the spectrophotometric determination in which non-aqueous solvents like methanol [34] and DMF [35] are used while the second type uses aqueous buffer (pH 11) and the resulting purple colour formed was measured at 520 nm [32]. The third group include reduction reaction in which a purssian blue complex is obtained with maximum absorbance at 728 nm [31]. But the determinations reported earlier are less sensitive.

ISSN-2394-5125 VOL 03, ISSUE 02, 2016

Sulfanilamides are commonly used as antibacterial that are aniline substituted sulfamides. Though, a large number of sulfanilamide derivatives synthesized are reported in the literature, only about two dozen have been used in clinical practice [36]. Despite the toxicity observed with some patients and the existence of sulfanilamide-resistant bacterial strains the use of these drugs in combination especially sulfonamide-trimethoprims has been extensively used for opportunistic infections in patients with AIDS, pneumonia (Pneumocystiss carinil) treatment and prophylaxis, cerebral toxoplasmosis treatment and prophylaxis, urinary tract infection and burn therapy [37-39].
Sulfanilamide (SAA), sulfadoxine (SDX) and sulfamethoxazole (SMX) are the chemicals which contain aromatic primary amino group. SDX is a long-acting sulfanilamide; used in the treatment of various types of infections. It exhibits synergistic effect with pyrimethamine, which acts against folate metabolism at different points of the metabolic cycle. SMX is commonly used to treat uncomplicated urinary tract infection, more particularly those caused by Escherichia coli.
This paper is an attempt to develop simple, sensitive, rapid and reliable spectrophotometric methods for the determination of curcumin. Survey of literature revealed that no sulfa drugs and their derivatives have been used for the spectrophotometric determination of curcumin. The methods reported here in involve coupling of diazotized sulfanimides with curcumin in alkaline medium to produce yellow colour.

Experimental

Apparatus

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

Reagents

Curcumin (Vittal Mallya Scientific Research Foundation, India), sulfanilamide (SAA), sulfadoxine (SDX) and sulfamethoxazole (SMX) (Glaxo Smithkline Pharmaceuticals, India was used. All other chemicals and solvents used were of analytical reagent grade. Double distilled water was used throughout. Curcumin (100 mg) was dissolved in isopropyl alcohol in a 100-ml volumetric flask and made up to the mark. The stock solution was further diluted with isopropyl alcohol to get solutions of required strength. Aqueous solutions of 1.0% (*w/v*) sodium nitrite, 1.0% (*w/v*) sulphamic acid and 0.5N sodium hydroxide solutions were prepared in distilled water. Aqueous solutions of 0.25% (*w/v*) sulfanilamide, sulfadoxine and sulfamethoxazole were prepared in distilled water. Ten ml of 2N hydrochloric acid was added during the preparation of sulfadoxine and sulfamethoxazole to improve its solubility.

Procedures

Two ml of SAA, SDX or SMX, 1.0ml each of sodium nitrite and sulphamic acid solution were transferred into a series of 25ml-calibratedflask. To this aliquot of standard solution of curcumin were added and 1.0 ml of sodium hydroxide was added and the contents were shaken well, and diluted to mark using distilled water. The absorbance was then measured against the corresponding reagent blank at 425 nm. The optical characteristics are shown in Table 1.

Table 1: Optical characteristics for the determination of curcumin

Parameters	**SAA**	**SDX**	**SMX**
Colour	Yellow	Yellow	Yellow
λ (nm)	425	425	425
Stability (h)	24	24	24
Beer's law (μg ml^{-1})	2.0 – 15.0	2.5 – 30.0	1.0 – 13.0
Recommended concentration (μg ml^{-1})	7.0	14.0	7.0
Molar absorptivity (L mol^{-1} cm^{-1})	$2.22x\ 10^4$	$0.65\ x\ 10^4$	$1.11\ x\ 10^4$
Sandell's sensitivity (μg cm^{-1})	0.038	0.089	0.019
Regression equation*			
Slope (a)	0.1794	0.345	0.2401
Intercept (b)	0.008	0.0023	0.0104
Correlation coefficient	0.9976	0.987	0.9881
R.S.D. % **	± 0.70	± 1.12	± 0.54

*y=ax+b where x is the concentration of curcumin in μg ml^{-1}.
**relative standard deviation (n=5),
SAA: sulfanilamide, SDX: sulfadoxine, SMX:sulfamethoxazole

Results and discussion

For the activation of the substrates pH of the media is of paramount importance. For phenols, alkaline medium is recommended because phenols themselves are not active enough for the diazotisation reaction. Nevertheless, there is a risk of unstable derivatives and large values of blank due to the process called hydroxy-de-diazonization [40] which reacts with excess of the reagent in basic medium.

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The proposed sulfa drugs namely; SAA, SDX and SMX containing aryl primary amino group undergo diazotization reaction using sodium nitrite solution to produce diazonium group which reacts with curcumin in sodium hydroxide medium to produce a yellow colour dye. The method involves the coupling of the diazotized sulfa drug with curcumin to produce a yellow-coloured product with maximum absorption at 425 nm. The NH group of the sulfa drug gets readily diazotized during the diazotisation process to produce diazonium group which reacts with curcumin in sodium hydroxide medium to produce a yellow-coloured dye.

Spectral characteristics

A yellow-coloured product with maximum absorption at 425 nm was formed when sulfanilamide, sulfadoxine, sulfamethoxazole reacted with curcumin in sodium hydroxide medium.

Optimization of analytical variables

The choice of an appropriate solvent/medium has profound influence on the sensitivity and reproducibility of the results. Full colour development and maximum sensitivity were achieved when the reaction was carried out in an alkaline medium. It was found that sodium nitrite (1.0% w/v) in the range 0.5-2.5 ml, sulphamic acid (1.0% w/v) in the range 0.5-2.0 ml and 0.5 N sodium hydroxide 0.5-1.5 ml were sufficient to get reproducible results. Hence, sodium nitrite, sulphamic acid and sodium hydroxide each at 1.0 ml was recommended. Similar experiments were carried out to know the amount of SAA, SDX and SMX. It was found that 1.0-3.0 ml (0.25% w/v) of SAA, SDX and SMX were found to give maximum colour intensity. Hence, 2.0 ml each of SAA, SDX and SMX were found appropriate.

Table 1 shows the linear calibration ranges and equation parameters for these methods. Separate determinations at different concentrations of each reagent gave a coefficient of variation not exceeding 2%.

Interference

The interference if any, by various substances was studied as per the procedure. Excipients such as glucose, lactose, dextrose, starch, sodium alginate and sodium lauyrl sulphate did not interfere in the determination, while vitamin C was found to interfere (Table 2).

Table 2: Recovery of curcumin in the presence of excipients and other substances using sulfanilamide

Material	Amount(mg)	% Recovery of curcumin* $\pm$ RSD**
Gluocose	50	97.5 $\pm$ 0.99
Lactose	50	99.3 $\pm$ 0.67
Dextrose	50	99.6 $\pm$ 0.45
Starch	50	100.3 $\pm$ 0.60
Sodium alginate	50	98.7 $\pm$ 0.97
Sodium lauryl sulphate	50	99.5 $\pm$ 0.64
Vitamin C	10	>50 < 60©

*7.0 µg ml^{-1} of curcumin taken, **relative standard deviation (n=5),
©erratic values

Conclusion

The procedures described in this paper are the first-ever use of sulfa drugs containing amino group as spectrophotometric reagents for the determination of curcumin. Two important dimensions of this study include the success in finding new spectrophotometric reagents amongst the available myriad molecules in the field of pharmaceuticals which has a variety for the functional groups and molecular structure. Second, it will open up a new area of research on the dyes produced in the nation of curcumin with sulfa drugs.

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