Innovative Spectrophotometric Methods for the Determination of Anacardic Acid

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***Abstract* - Anacardic acid, a phenolic compound is found in cashew nut shell liquid (CNSL) a by-product of cashew industry. It holds considerable promise because of its large availability in tropical areas, low cost, biodegradability and structural characteristics. A study was made of the application of diazotization-coupling spectrophotometric technique for the determination of anacardic acid, using sulfanilamide (SAA), sulfadoxine (SDX) and sulfamethoxazole (SMX) the widely used sulfa drugs as coupling agents. The methods are based on the interaction of diazotized drugs with anacardic acid in alkaline medium to produce a orange red color product having maximum absorption at 480 nm. The color developed was stable for 4 h at 27oC. Beer's law was obeyed in the concentration range 1.6-12.8 µgml-1 for SAA and 1.0-14.4 µgml-1 for SDX and SMX at the wavelength of maximum absorption at 480 nm. The method was also successfully tried for the determination of anacardic acid in presence of various anions and cations, which did not interfere in the proposed methods.**

***Keywords*: Anacardic Acid, Sulfanilamide, Sulfadoxine, Sulfamethoxazole, Spectrophotometry**

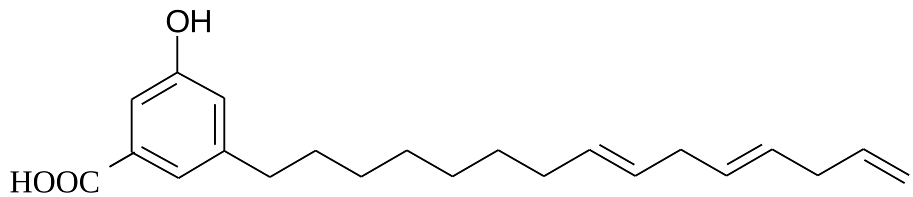
I. INTRODUCTION

Sulfanilamides are analogues of *p*-aminobenzoic acid and are known since 1932. They differ only slightly in there antimicrobial activity but varies greatly in their pharmacokinetic properties. Though, a large number of synthesized sulfanilamide derivatives are reported in the literature, only about two dozens of them have been used in clinical practice [1]. Sulfanilamide (SAA), sulfadoxine (SDX) and sulfamethoxazole (SMX) are the chemicals which contain aromatic primary amino group. SAA exhibits synergistic effect with pyrimethamine, which acts against folate metabolism at different points of the metabolic cycle. SDX is a long-acting sulfanilamide used in the treatment of various types of infections. SMX is commonly used to treat uncomplicated urinary tract infection, more particularly those caused by *Escherichia coli* [2-4].

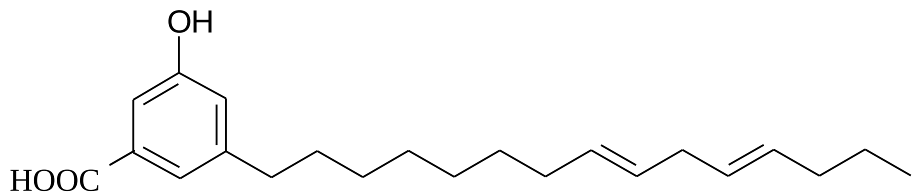
Anacardic acid (3-pentadecenyl phenol) is a phenolic compound with C_{15} aliphatic chains in the meta position. It is a mixture of saturated and unsaturated (mono-, di- and tri-) compounds [5] (Fig.1) commonly found in cashew nut shell liquid (CNSL), an alkyl phenolic oil which constitutes 25% of the total weight of cashew nut (*Anacardium occidentale)* a well known species of the anacardiaceae family [6]. CNSL is obtained as a by-product of cashew industry, and is an important source of unsaturated hydrocarbon phenol [7].

Anacardic acid holds considerable promise because of its structural characteristics [8], large availability in tropical areas, low cost and biodegradability [9]. The non-linear structure, unsaturation in the alkyl chain and substitution to phenolic group opens up new vistas in the innumerable applications in dyestuffs, foods, flavors, ion exchange resins, paints, plasticizers and polymers [10].

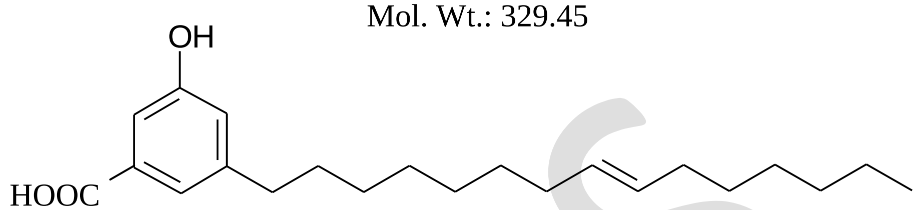
Anacardic acid inhibits enzymes such as prostaglandin synthase [11], tyrosinase [12] and lipoxygenase [13]. It is also known to exhibit antitumor [14] antimicrobial [15] and antiacne [16] properties. Anacardic acid exhibits thermostability of the carboxylic group and there is a tendency to get converted to cardanol [17].



Mol. Wt.: 327.43



Mol. Wt.: 329.45



Mol. Wt.: 331.46

Mol. Wt.: 333.48

Fig. 1: Structures and molecular weights of anacardic acid

The possible growth in the commercial applications of anacardic acid has encouraged developing sensitive, rapid and reliable methods for its determination. Survey of the literature revealed that no spectrophotometric method has been reported so far for its determination. Hence, this is the first report on visible spectrophotometric methods for the determination of anacardic acid.

The work described here is first-ever study and forms part of a systematic investigation to develop new spectrophotometric methods for agro products – a field of paramount importance due to easy biodegradability and use of agricultural waste and by-products, in place of toxic chemicals – an area of current interest in environmental management.

II. MATERIALS AND METHODS

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

2) Reagents: Anacardic acid (Vittal Pharma, India), sulfanilamide (SAA), sulfadoxine (SDX) and sulfamethoxazole (SMX) (Glaxo Smithkline Pharmaceuticals, India), sodium nitrite, suplhamic acid and sodium hydroxide (Ranbaxy, India) were used. All other chemicals and solvents used were of analytical reagent grade. Double distilled water was used throughout. Aqueous solutions of 0.5% (*w/v*) sodium nitrite, 0.5% (*w/v*) sulphamic acid, 1N sodium hydroxide, 0.25% (*w/v*), sulfanilamide, sulfadoxine and sulfamethoxazole were prepared in distilled water. Five ml of 2N hydrochloric acid was added during the preparation of sulfadoxine and sulfamethoxazole to improve its solubility. Anacardic acid (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.

3. Procedure: One ml each of SAA, SDX or SMX, and 1ml each of sodium nitrite and sulphamic acid were transferred into a series of 25 ml-calibrated flasks. Aliquots of standard solution of anacardic acid were added to this and 1ml of sodium hydroxide was added and the contents were shaken well till the effervescence stopped and kept aside for 10 to 15 min at room temperature, diluted up to the mark using distilled water. The absorbance was measured against the corresponding reagent blank at 480 nm.

III. RESULTS AND DISCUSSION

Weak electrophilic diazotized arylamines couple with strong activated substrates to give colored azo dyes. When the substrates are also arylamines [18, 19] a weak acidic medium is recommended for coupling. But, a basic medium is required with phenols, because only free amines and phenolate ions are sufficiently activated [20]. Unfortunately, unstable derivatives and large values of blank solutions are usually obtained. These effects are caused by the hydrolysis of dizonium ion of the reagent used for coupling to give a phenol in a process called hydroxy-de-diazonization [21] which reacts with excess of reagent in basic medium.

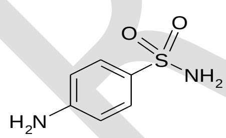
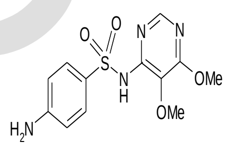
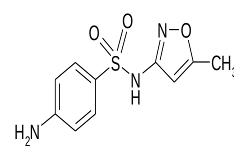
Anacardic acid contains hydroxyl groups and the proposed methods involve coupling of diazotized sulfa drug with anacardic acid in basic medium to produce an orange red color product with maximum absorption at 480 nm. The factors affecting the color development such as reproducibility, sensitivity and adherence to Beer's law were investigated separately for each reagent.

1. Spectral characteristics: An orange red colored product with maximum absorption at 480 nm was formed when sulfanilamide, sulfadoxine, sulfamethoxazole reacted with anacardic acid in sodium hydroxide medium.

2. Optimization of analytical variables: It was found that sodium nitrite 1.0% (*w/v)* for SAA and 0.5% (*w/v)* for SDX and SMX in the range 1-3 ml, sulphamic acid 0.5% (*w/v*) for SAA, SDX and SMX in the range 1-2 ml and 1N sodium hydroxide 0.5-3 ml for SAA,SDX and SMX gave reproducible results. Hence, sodium nitrite, sulphamic acid and sodium hydroxide each at 1.0 ml were found optimum. Similar experiments were carried out to know the amount of SAA, SDX and SMX. It was found that 1.5-3 ml of 0.25% (*w/v*) SAA and 1.0-3.0ml of SDX and SMX were found to give maximum intensity. Hence, 2 ml of SAA, and 1 ml for 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%.

TABLE 1
OPTICAL CHARACTERISTICS FOR THE DETERMINATION OF ANACARDIC ACID

Parameters		 SAA	 SDX	 SMX
Colour		Orange red	Orange red	Orange red
λ_{max} (nm)		480	480	480
Stability (h)		4	4	4
Beer's law range (μg ml^{-1})		1.6-12.8	1.0-14.4	1.0-14.4
Recommended concentration	(μg ml^{-1})	6.4	8	8
Molar absorptivity	(L mol^{-1}cm^{-1})	1.9 x 10^4	1.8 x 10^4	1.9 x 10^4
Sandel's sensitivity	(μg cm^{-2})	0.00639	0.00413	0.00319
Regression equation*				
Slope (a)		0.0619	0.044	0.0377
Intercept (b)		0.0376	0.0246	0.3752
Correlation coefficient		0.99	0.990	0.961

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

SAA: sulfanilamide, SDX: sulfadoxine, SMX: sulfamethaxazole

3. Interference: The effect of various anions and cations on the determination of anacardic acid was studied as per the proposed procedures and the results are presented in Table 2 and 3. In general, 100 mg of the respective salt was added individually to aliquots containing 5.0 µg ml^{-1} of anacardic acid. The results showed that the methods are free from interference by any of the anions and cations, studied.

Table 2
Effect of Anions on the determination of Anacardic Acid

Anion salt added	Quantity added (mg)	% Recovery ± RSD**
Ammonium phosphate	100	99.4 ± 0.92
Calcium carbonate	100	100.6 ± 1.11
Potassium bromate	100	101.2 ± 0.96
Potassium chloride	100	100.4 ± 0.78
Potassium iodate	100	99.5 ± 0.74
Potassium sulphate	100	98.8 ± 1.01
Sodium fluoride	100	101.4 ± 1.00
Sodium nitrate	100	97.8 ± 0.90
Sodium phosphate	100	99.7 ± 0.78
Sodium sulphate	100	101.6 ± 0.84

**Relative standard deviation, n=5

Table 3
Effect of Cations on the determination of Anacardic Acid

Cation salt added	Quantity added (mg)	% Recovery ± RSD**
Barium sulphate	100	98.4 ± 0.98
Cadmium sulphate	100	99.67 ± 1.04
Copper sulphate	100	99.32 ± 1.08
Lead nitrate	100	100.3 ± 0.84
Magnesium sulphate	100	101.1 ± 0.72
Manganese sulphate	100	99.36 ± 0.68
Potassium dichromate	100	99.2 ± 0.76
Strontium nitrate	100	98.6 ± 1.02
Tin chloride	100	100.8 ± 0.90
Zinc sulphate	100	101.4 ± 0.84

**relative standard deviation, n=5

IV. CONCLUSION

The phytochemicals has produced a profound effect on the Western medical system, which is now trying to acknowledge methods of healing that was in existence 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 in quality control. In the present context, determination or estimation of anacardic acid is of paramount importance. Simple methods based on spectrophotometry may dominate as analytical tool for the evaluation of phytochemicals. Our methods are a step forward towards this direction.

The proposed first-ever spectrophotometric methods for determination of anacardic acid have adequate sensitivity and accuracy. Their analytical characteristics such as simplicity, selectivity and stability are far superior to other existing spectrophotometric methods reported for agriculture products.

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