

New and Sensitive Spectrophotometric Method for the Determination of Cardanol- An Agricultural By-product

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Abstract

Cardanol, 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. Simple, sensitive, selective, rapid and reliable spectrophotometric method for the determination of cardanol, an agriculture by-product has been developed. The method is based on the reaction of cardanol with an electrophilic coupling reagent, 3-methyl-2-benzothiazoline hydrazono hydrochloride hydrate (MTH) in presence of iron (III) in hydrochloric acid medium to produce a red colour product having maximum absorbance at 480 nm. The method obeys Beer's law. As many as ten independent anions and cations did not interfere with the determination.

Key words: Cardanol, MBTH, Agriculture by-product, Cashew nut, Spectrophotometry

Cardanol (3-pentadecenyl phenol) is a phenolic compound with Cis aliphatic chains in the meta position. It is a mixture of saturated and unsaturated (mono-, di- and tri-) compounds [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 [2]. CNSL is obtained as a by-product of cashew industry, and is an important source of unsaturated hydrocarbon phenol [3]. Cardanol holds considerable promise because of its structural characteristics, [4], large availability in tropical areas, low cost and biodegradability [5]. The non-linear structure, unsaturation in the alkyl chain and substitution to phenolic group opens up new vistas in the innumerable applications in dyestuff, food, flavour, ion exchange resins, paints, plasticizers and polymers [6]. Significant studies have also been made in the technological application of cardanol and its derivative as pesticides [7] and surface-active agents [7] besides, in ceramics [4] and composites [5].

The phenomenal growth in the commercial applications of cardanol has encouraged the authors to develop sensitive, rapid and reliable methods for its determination. Survey of the literature revealed that no analytical method has been reported so far for its determination. The work described in this paper 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.

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

Reagents

Cardanol from Vittal Mallya Scientific Research Foundation, India, 3-methyl-2-benzothiazoline hydrazono hydrochloride hydrate (MBTH) (Sigma, USA), iron (III) chloride, (BDH, India), isopropyl alcohol, hydrochloric acid (Ranbaxy, India) was used. Alcohol was distilled before use and double distilled water was used throughout. Cardanol (100 mg) was dissolved in isopropyl alcohol in a 100-ml volumetric flask and made up to mark. This stock solution was diluted with isopropyl alcohol to get solutions of required strength. Aqueous solutions of MBTH (0.1% w/v), iron (III) chloride (0.1% w/v) and 5N (v/v) hydrochloric acid were prepared. Solution of MBTH was stored in amber bottle to protect from sunlight.

Procedures

Aliquots of standard solutions of cardanol, 1.0 ml of MBTH, 1.0 ml of iron (III) chloride and 1.0 ml of hydrochloric acid were transferred into a series of 10-ml calibrated flasks. The contents were mixed well and allowed to stand for 10 min. It was made up to 10-ml mark using alcohol. The absorbance was measured at 480 nm against the corresponding reagent blank and calibration graphs were constructed. The optical characteristics for the determination of cardanol are shown in (Table 1).

RESULTS AND DISCUSSION

The chemical reaction is the reduction of iron (III) chloride by MBTH, which subsequently couples with cardanol

MATERIALS AND METHODS

Apparatus

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in hydrochloric acid medium to form a red-coloured product having maximum absorption at 480 nm. MBTH is an electrophilic coupling reagent, which reacts with different types of organic compounds. The results of the reaction of organic compounds with MBTH have often been applied in analysis pertaining to biochemical [8], pharmaceutical [9-14], insecticides [15] and in flow injection analysis [16-17].

The choice of oxidizing agent depends on the reaction medium. Hexacyanoferrate (III) is a preferred choice in alkaline medium [18], while, iron (III) or cerium (IV) is used in acidic medium, because iron (III) is sensitive at pH >8 and forms a gelatinous precipitate of hydrated ferric oxide. While, cerium (IV) forms precipitate at pH >1. However, modification by making the reaction mixture alkaline only after preparing the coloured products in an acidic media [18] has been utilized in analytical practice.

The coloured products are the complexes formed between MBTH and the respective substrate, which are generally insoluble in water and readily soluble in organic solvents. The organic solvents as reported in the literature include, methanol, ethanol, isopropyl alcohol, dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO). In the present context the method is based on the reaction of MBTH with phenols [19] is interesting, because it is the prevalence of the polar structure of the product molecule which depends on the character of the reaction medium. The reactions take place in acid media and in the presence of an oxidizing agent. The order of mixing of the reactants is the sample, MBTH and oxidizing agent, which is common in all published procedures.

Addition of a few drops of 2N HCl is necessary to prevent precipitation of iron (III) as hydrated ferric oxide. Besides, the addition of hydrochloric acid will bring down the pH of the solution in slightly acidic range. Neutral ferric chloride free from hydrochloric acid is reported to give different colours, green, purple or blue which have been extensively exploited as a confirmatory test of phenols in organic chemistry [20]. The present work describes the details of the factors affecting the colour development, reproducibility, sensitivity and adherence to Beer's law.

Optimization of analytical variables

For a fixed concentration of cardanol, MBTH and hydrochloric acid the colour intensity remains constant with 0.5-2.0 ml 0.1% (w/v) of iron (III) chloride. Hence, for routine analysis 1.0 ml of iron (III) chloride was used. Similarly, the amounts of MBTH and hydrochloric acid required for constant colour intensity were also determined. It was found that 0.5-2.0 ml of 0.1% (w/v) of MBTH, 0.5-2.5 ml of 5N hydrochloric acid were required to get maximum colour intensity and stability. Hence, 1.0 ml each of MBTH and hydrochloric acid were found optimum to get reproducible results. The red colour development was slow when other acids like sulphuric acid, nitric acid, phosphoric acid and acetic acid were used.

Order of addition

The sequence of addition of cardanol, MBTH, iron (III) chloride and hydrochloric acid for the formation of the red complex was studied. The sequence of addition of reactants showed profound influence on the intensity and stability of the red colour as for sample addition in the order: (1) MBTH + iron (III) chloride + HCl + cardanol or (2) iron (III) + HCl + cardanol + MBTH + less intense and unstable colour, compared to (3) cardanol + MBTH + iron (III) + HCl which gave more intense and very stable colour.

Effect of solvents

The coloured product was developed at room temperature which was stable for 5 hours. The colour intensity decreased rapidly when diluted with water hence, the coloured product was diluted with ethyl alcohol. Isopropyl alcohol was the preferred solvent for preparing stock solution of cardanol 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 to ethyl alcohol and methyl alcohol. The solvents like DMF and DMSO were not chosen for the study because of their toxicity and high cost. Ethyl alcohol was preferred to methyl alcohol as it is nontoxic.

Calibration and spectral data

Molar absorptivity is an important parameter in UV-visible spectroscopic studies and it is a ratio of absorbance to concentration. Conversely, concentration is volume and molecular weight dependent. The red colour complexes obeyed Beer's law. The optical characteristics, such as optimum range, as evaluated from Ringbom plot, molar absorptivity, sandell's sensitivity, slope, intercept and correlation coefficient are shown in (Table 1). The values indicate moderate dynamic range and high sensitivity.

Table 1 Spectral data for the determination of cardanol using MBTH

Parameters	
Colour	Red
λ_{\max} (nm)	480
Stability (h)	5
Beer's law (ng ml ⁻¹)	1.0-18.0
Recommended drug concentration (µg ml ⁻¹)	4.0
Molar absorptivity (L mol ⁻¹ cm ⁻¹)	2.03 x 10 ⁴
Sandell's sensitivity (µg cm ⁻²)	0.014
Regression equation*	
Slope (a)	0.2860
Intercept (b)	0.0502
Correlation coefficient	0.9896
R.S.D. % **	±0.68

*y = ax + b where x is the concentration of cardanol in µg ml⁻¹

**relative standard deviation (n=5)

Table 2 Effect of anion on the determination of cardanol

Salt of the anion added	Salt added (mg)	% Recovery of cardanol* ± RSD**
Ammonium phosphate	100	100.8 ± 0.54
Calcium carbonate	100	98.9 ± 1.08
Potassium bromate	100	99.6 ± 0.86
Potassium chloride	100	98.6 ± 0.91
Potassium iodate	100	98.8 ± 0.52
Potassium sulphate	100	100.4 ± 0.75
Sodium fluoride	100	98.7 ± 0.98
Sodium nitrate	100	100.3 ± 0.86
Sodium phosphate	100	99.1 ± 0.45
Sodium sulphate	100	98.5 ± 0.71

*4.0 µg ml⁻¹ of cardanol

** relative standard deviation (n=5)

Interference

The effect of various anions and cations on the determination of cardanol was studied as per the proposed procedures and the results are presented in (Table 2-3). In

general, 100 mg of the respective salt was added individually to aliquots containing 4.0 $\mu\text{g ml}^{-1}$ of cardanol for Method A and B, respectively. The results showed that the methods are free from interference by any of the anions and cations, studied. However, cardol interfered in the determination.

Table 3 Effect of cation on the determination of cardanol

Salt of the cation added	Salt added (mg)	% Recovery of cardanol* \pm RSD**
Copper sulphate	100	99.4 \pm 0.61
Barium sulphate	100	99.5 \pm 1.08
Cadmium sulphate	100	99.1 \pm 0.82
Lead nitrate	100	100.6 \pm 0.76
Magnesium sulphate	100	99.4 \pm 0.61
Manganese sulphate	100	99.7 \pm 0.96
Potassium chromate	100	100.1 \pm 0.73
Strontium nitrate	100	98.9 \pm 0.82
Tin chloride	100	99.0 \pm 0.93
Zinc sulphate	100	100.3 \pm 0.59

*4.0 $\mu\text{g ml}^{-1}$ of cardanol

** relative standard deviation (n=5)

Applications

Five crude samples of cardanol procured from different sources were analysed by the conventional standard addition method. Each sample was analysed with five replicates and the result of RSD was within # 2%, and this margin of error is acceptable in all spectrophotometric determinations (Table 4).

CONCLUSION

One of the recent frontiers of sustainable development has been the utilization of agricultural by-products. Effective utilization of any product is based on its quality, which, in turn, depends on the analytical data. The procedure described in this paper is the spectrophotometric method which meets most of the demands of analytical chemists namely; selectivity, sensitivity, simplicity, rapidity, reliability and cost of analysis. In this method, it is necessary to use iron (III) as the oxidizing agent; the use of such simple reagents makes the procedure cost-effective. One of the important facts is that this study will open up a new area of research Further value-addition to this method can be achieved, if the procedure is combined with on-line or at-line system and this is currently under investigation.

Table 4 Cardanol content determined in various crude samples

Sample	Amount of cardanol found by proposed method (g)	Cardanol added to the same sample (g)	Amount of cardanol found by difference (g)	Recovery **%
1	0.0976	0.100	0.0982	98.2
2	0.0994	0.100	0.0992	99.0
3	0.0980	0.100	0.1010	101.0
4	0.1020	0.100	0.0989	98.9
5	0.0974	0.100	0.1010	101.0

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