Determination of Agricultural by-product: Cardanol

Syeda Ayesha

Department of Chemistry, G.F.G. College, Kuvempu Nagara, Mysore-570023, India

Abstract: Simple, sensitive, selective, rapid and reliable methods for the spectrophotometric determination of cardanol has been worked out. The methods are based on the reaction of cardanol with folin ciocalteu (FC), an electrophilic coupling agent in presence of iron (III) in mild hydrochloric acid medium. The bluish –green complex shows maximum absorbance at 660nm. The colour complex can be extracted into chloroform. The methods obey Beer's law.

Keywords: Cardanol, Cashew nut, Anacardium occidentale , Spectrophotometry, Folin ciocalteu

1. INTRODUCTION

Cardanol holds considerable promise because of its abundant availability in tropical areas, low cost, biodegradability [1] and structural characteristics [2]. Cardanol found in cashew nut shell liquid (CSNL), a by-product of cashew industry. CSNL is an alkyl phenol and which constitutes 25% of the total weight of cashew nut [3].

Cardanol is a renewable raw material derived from a byproduct of cashew nut processing industry: Cashew Nut Shell Liquid (CNSL). Cardanol is a rich mixture of non-isoprenoic phenolic compounds that is a valuable raw material for generating a variety of soft nanomaterials such as nanotubes, nanofibers, gels and surfactants. The name of the substance is derived by contraction from the genus Anacardium, which includes the cashew tree, Anacardium occidentale.

Among the renewable resource materials, cashew nut shell liquid (CNSL) is considered as an important starting material due to its unique structural features, abundant availability and low cost. A large number of chemicals and products have been developed starting from CNSL by taking advantage of the three reactive sites, namely, phenolic hydroxyl, aromatic ring and unsaturation(s) in the alkenyl side chain. Increasing attention is paid to promising cardanol-based products that could be have potential interest in industry, such as epoxy and acrylic monomers, plasticizers and surfactants.

Cardanol (3-pentadecenyl phenol) is a phenolic compound with C15 aliphatic chains in the meta position. It is a mixture of saturated and unsaturated (mon-, di- and tri-) compounds [4]. The non-linear structure, unsaturation in the alkyl chain and substitution in phenolic group opens up new vistas in its innumerable application including dyestuff, food, flavor, ion exchange resin, paints, plasticizers and polymers [5]. Significant studies have also been made in the technological applications of cardanol and its derivatives as pesticides [6], surface-active agents [6], ceramics [2] and composites [1].

The work described here is a novel and highly sensitive method for the determination of cardanol which is based on the reaction involving the use of iron (III) salts in the presence of electrophilic coupling reagent folin ciocalteu (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.

2. MATERIALS AND METHODS

2.1 Apparatus

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

2..2 Reagents

Cardanol (Vittal Mallaya Scientific Research Foundation, India), folin ciocalteu (FC),(BDH, India) and iron (III) chloride (BDH, India) were used. All other chemicals and solvents were of analytical grade. Double distilled water was used throughout. Cardanol (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 folin ciocalteu, (0.1% *w/v*), iron (III) chloride (0.5% *w/v*) containing few drops of 2N (*v/v*) hydrochloric acid was prepared. Solution of folin ciocalteu (FC) was 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 (Method A)

Aliquots of standard solutions of cardanol were transferred into 10-ml calibrated flasks. 1.0 ml of folin ciocalteu (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 up to mark using alcohol. The absorbance was measured at 660nm against the corresponding reagent blank and calibration graphs was constructed.

(ii)Extractive spectrophotometry (Method B)

Appropriate volume of standard cardanol solution, 1.0 ml of folin ciocalteu (0.1% *w/v*) and 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 up to mark using chloroform in 10-ml calibrated flask. The absorbance was measured at 660nm against the corresponding reagent blank and calibration graphs was constructed. The optical characteristics determined are presented in Table 1.

3. RESULTS AND DISCUSSION

Cardanol is a phenolic lipid obtained from anacardic acid, the main component of cashew nutshell liquid (CNSL), a byproduct of cashew nut processing. Cardanol finds use in the chemical industry in resins, coatings, frictional materials, and surfactants used as pigment dispersants for water-based inks. It is used to make phenalkamines, which are used as curing agents for the durable epoxy coatings used on concrete floors.[7]

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[8]. 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 such as hydroxylamine and guanidine[9]. 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[10].

Table 1: Spectral data for the determination of cardanol using folin ciocalteu as electrophilic coupling reagent

Parameters	DS (Method A)	ES (Method B)
Colour	Bluish green	Bluish green
λ_{max} (nm)	660	660
Stability (h)	6	6
Beer's law (ng ml^{-1})	0.2-7.0	0.1-6.0
Recommended drug concentration (ng ml^{-1})	4.0	3.0
Molar absorptivity (L mol^{-1} cm^{-1})	2.67×10^4	4.65×10^4
Sandell's sensitivity (µg cm^{-2})	0.006	0.005
Regression equation*		
Slope (a)	0.0554	0.1230
Intercept (b)	0.0564	-0.064
Correlation coefficient	0.9775	0.9432

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

3.1 Reaction mechanism

The chemical reaction in the procedure described for the spectrophotometric determination of cardanol involves the reduction of iron(III) chloride by folin ciocalteu which subsequently couples with cardanol to form a bluish-green product having a maximum absorption at 660nm. The colour intensity remains constant for 6 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 660nm was formed when cardanol was allowed to react with iron(III) chloride in the presence of folin ciocalteu in mild hydrochloric acid medium.

3.3 Optimization of analytical variables

For a fixed concentration of cardanol and folin ciocalteu, 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 folin ciocalteu required for constant colour intensity. It was found that 0.5-4.0 ml of folin ciocalteu (0.1% *w/v*) was required to provide maximum colour intensity and stability. Hence, 1.0 ml of 0.1% *(w/v)* of folin ciocalteu was found to be optimum to get reproducible results.

3.4 Order of addition

The sequence of addition of cardanol, folin ciocalteu 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 intensity and the stability of the colour, for example, (1) folin ciocalteu +iron (III)chloride +cardanol and (2) iron (III) chloride+ cardanol+ folin ciocalteu gave less intense and unstable colour. While, the order: (3) cardanol + folin ciocalteu +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 6 h. Isopropyl alcohol was the preferred solvent for preparing stock solution of cardanol 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 cardanol. Conversely, methyl alcohol and ethyl alcohol have profound influence and enhances the stability of the colour and for routine 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's sensitivity, slope, intercept, correlation coefficient are shown in Table 1.

3.7 Interference

The effect of various anions and cations on the determination of cardanol 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 cardanol with iron(III) and folin ciocalteu for method A and B, respectively.

Table 2: Effect of anion on the determination of cardanol

Salt of the anion added	Salt added mg	% Recovery of cardanol* ± RSD**	
		Method A	Method B
Ammonium tartarate	100	99.2 ± 0.86	99.6 ± 0.68
Calcium carbonate	100	98.3 ± 1.02	98.4 ± 0.78
Potassium bromate	100	98.9± 0.68	98.2 ± 0.64
Potassium chloride	100	100.2 ± 0.82	99.4 ± 0.96
Potassium iodate	100	99.2 ± 0.80	97.6 ± 0.54
Potassium sulphate	100	99.6 ± 0.94	98.2 ± 1.03
Sodium fluoride	100	99.8 ± 0.90	99.4 ± 1.04
Sodium nitrate	100	99.2 ± 0.80	99.6 ± 0.54
Sodium phosphate	100	99.6 ± 0.94	99.6 ± .080
Sodium sulphate	100	100.8 ± 1.02	98.3 ± 0.78

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

Table 3: Effect of cation on the determination of cardanol

Salt of the anion added	Salt added mg	% Recovery of cardanol* ± RSD**	
		Method A	Method B
Ammonium molybdate	100	100.6 ± 0.80	98.4 ± 1.04
Barium sulphate	100	98.8 ± 0.76	99.8 ± 0.98
Cadmium sulphate	100	98.6 ± 0.56	99,6 ± 0.86
Lead nitrate	100	98.6 ± 0.88	98.6 ± 0.68
Magnesium sulphate	100	99.4 ± 0.72	98.4 ± 0.84
Manganese sulphate	100	99.2± 0.84	100.4 ± 1.06
Potassium chromate	100	99.3± 1.06	99.4 ± 1.02
Strontium nitrate	100	99.4 ± 1.06	98.2 ± 1.02
Tin chloride	100	101.8 ± 0.98	99.6 ± 0.94
Zinc sulphate	100	100.2 ± 0.78	97.6 ± 0.54

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

4. CONCLUSION

In the present context, determination or estimation of cardanol is 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 cardanol. Their analytical characteristics such as sensitivity, selectivity and stability excelled other existing spectrophotometric methods. Visible spectrophotometer is easily available in most laboratories for routine determination of curcumin, especially in developing countries

REFERENCES

1.Maffezzoli A. Calo E. Zurlo G. Mele A Tarzia, and Stifani D. (2004) “Cardanol based matrix biocomposites reinforced with natural fibres” Composites Science and Technology,64, Pp.839-845.

2. Prabhakaran K. Asha N and Pavithran C. (2001) “Cardanol as a dispersant plasticizer for an alumina/ toluene tape casting slip” Journal of the European Ceramic Society, 21, Pp. 2873-2878.

3. Tyman J H P. (1996) “Synthetic and Natural Phenols”Elseveir, Asterdam.

4. Tyman J H P. (1979) “Non-Isoprenoid long chain phenols”Chem. Soc, Rev., 8, Pp.499-537.
5.Cashew Export Promotion Council, Cashew Nut Shell Liquid, Extraction and uses- A Survey of World Patents up to 1976: Regional Research Laboratory, Hyderabad, India, Cashew Export Promotion Council: Cochin, India (1978).

6. Kumar P P. Paramashivappa R. Vithyaathil P J. Rao P V S. and Asha A S. (2002) "Process of isolation of cardanol from technical cashew (Anacardium occidentale L) nut shell liquid "J. Agric.Food Chem.,50, Pp.4705-4708.

7.Tullo A H. (2008) "A Nutty Chemical" Chemical and Engineering News. 86 (36), Pp. 26–27.

8.Singleton. Vernon, L. Orthofer. Rudolf. Lamuela-Raventós. Rosa, M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, 299:152–178.

9. Ikawa, M. Schaper, T D. Dollard, CA. Sasner, JJ .2003. Utilization of Folin–Ciocalteu phenol reagent for the detection of certain nitrogen compounds. J. Agric. Food Chem. 51 (7): 1811–1815.

10. Everette Jace, D. Bryant, Quinton M..Green, Ashlee M. Abbey, Yvonne A. Wangila, Grant W. Walker, Richard B. 2010. Thorough Study of Reactivity of Various Compound Classes toward the Folin−Ciocalteu Reagent. J. Agric. Food Chem. 58 : 8139–8144.