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# Novel, Simple, Sensitive and Rapid Spectrophotometric Methods For the Determination of Cardol using Sulfanilamides as New Class of Coupling Reagents

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## ABSTRACT

***First-ever three spectrophotometric methods using sulfanilamide (SAA), sulfadoxine (SDX) and sulfamethoxazole (SMX), the widely used sulfa drugs as new class of coupling agents for the spectrophotometric determination of cardol, a phenolic compound found in cashew nut shell liquid, a by-product of cashew industry is proposed. The methods are based on the interaction of diazotized sulfa drugs with cardol to produce an orange yellow colored product with a maximum absorption at 440 nm. The color developed was stable up to 6 h. The methods obey Beer's law. The methods can be successfully employed for the determination of cardol in presence of anions and cations, which do not interfere in the methods.***

***Key words: Cardol, Diazotization, Sulfanilamide, Sulfadoxine, Sulfamethoxazole, Spectrophotometry***

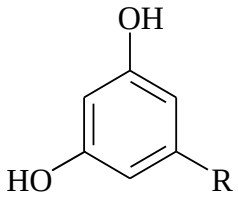
## I. INTRODUCTION

The world economy has created a network of dependency in fossil based energy and materials which have resulted in resurgence of new concepts for the possibilities to reduce this dependency in 21st century. Extensive studies are being carried out in search of replacing fossil resources due to its limitations and disadvantages, such as volatile prices and effects harmful to the environment. The Cashew Nut Shell Liquid (CSNL) is a versatile raw material with wide applications in the form of surface coatings, paints and varnishes, as well as the production of polymers. CSNL is alkyl phenolic oil which constitutes 25% of the total weight of cashew nut (Anacardium occidentale). The chemical constituents of CNSL become promising in the development of new materials components. Once separated, CNSL can be used in the research and development of additives, surfactants, pharmaceuticals, pesticides, polymers, resins and others. The CNSL is considered a by-product of the cashew agribusiness, having very low value. The liquid is a source of phenolic non-isoprenoid lipids of natural origin containing anacardic acid, cardanol, cardol and isomers (Attanasi et al., 2006; Kumar et al., 2002; Tyman and Kiong, 1978; Oltremare Indústria, 1979; Sadavarte et al., 2013; Mwaikamboa and Ansellb, 2003; Pathak and Rao, 2006; Agarwal and Dolui, 1995; Chand et al., 1986 and Lubi, 2007).

Cardol holds considerable promise because of its abundant availability in tropical areas, low cost, biodegradability (Maffezzoli et al., 2004) and structural characteristics (Prabhakaran et al., 2001and Tyman, 1996). Cardol is a mixture of saturated and unsaturated (mono-, di- and tri) compounds (Tyman, 1979) (Figure 1). The non-linear structure, unsaturation in the alkyl chain and substitution to phenolic group opens up new vistas in its innumerable applications including dyestuffs, foods, flavors, ion exchange resins, paints, plasticizers and polymers (Cashew Export, 1978).Significant studies have been made in the technological applications of cardanol and its derivatives as pesticides (Kumar et al., 2002), surface-active agents (Kumar et al., 2002), in ceramics

(Prabhakaran et al., 2001) and composites (Maffezzoli et al., 2004). Cardanol is another phenolic compound present along with cardol in the CSNL with one OH group. Since cardol has two OH groups it is likely that the applications of this compound will be more versatile than its counter part cardanol.




1. R=8Z, 11Z, 14 Pentadecatrienyl
   Mol. Wt.:299.43
2. R=8Z, 11Z Pentadecadienyl
   Mol. Wt.:301.45
3. R-8Z Pentadecenyl
   Mol. Wt.:303.46
4. R=Pentadecyl
   Mol. Wt.:305.43

**Figure 1. Structure of Cardol**

Sulfanilamide are commonly used as antibacterial. Though, a large number of sulfanilamide derivatives synthesized are reported in the literature, only few have been used in clinical practice (Northey, 1948). Sulfonamide-trimethoprims has been extensively used for opportunistic infections in patients with AIDS, pneumonia (Pneumocystis carinii) treatment and prophylaxis, cerebral toxoplasmosis treatment and prophylaxis, urinary tract infection and burn therapy (Mandell and Patri, 1996; Jewetz, 1995 and FDA Drug 1980). Sulfanilamide (SAA), sulfadoxine (SDX) and sulfamethoxazole (SMX) are the chemicals which contain aromatic primary amino group.

This paper is an attempt to develop simple, sensitive, rapid and reliable spectrophotometric methods for the determination of newly introduced agriculture by-product by exploring wide range of pharmaceuticals as new coupling agents. Survey of literature revealed that no spectrophotometric methods have been reported for the spectrophotometric determination of cardol. The methods reported here involve coupling of diazotized sulfanilamide with cardol in alkaline medium to produce yellow color. The proposed methods have distinct advantages of sensitivity and stability. Besides, the methods do not require heating or distillation and exhibit reliability due to reproducibility.

## II. MATERIALS AND METHODS

**Instrument:**

All absorbance measurements were recorded on UV-Visible spectrophotometer UVIIDEC-610 type with 1.0-cm matched cell (Jasco, Tokyo, Japan) was employed for measuring the absorbance values.

**Materials:**

Cardol (Vittal Mallya Scientific Research Foundation, India), sulfanilamide (SAA), sulfadoxine (SDX) and sulfamethoxazole (SMX) (Glaxo Smithkline Pharmaceuticals, India), sodium nitrite, suplhamic acid, sodium hydroxide (Ranbaxy, India) were used. All other chemicals and solvents used were of analytical reagent grade. Double distilled water was used throughout.

Cardol (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, 0.5% (w/v) sulphamic acid and 1.0 N sodium hydroxide solutions were prepared in distilled water. Aqueous solutions of 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 the solubility.

**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 cardol and 1ml of sodium hydroxide were added and the contents were shaken well, till the effervescence stopped and diluted up to the mark using distilled water. The absorbance was measured against the corresponding reagent blank at 440 nm.

## III. RESULTS AND DISCUSSION

Aromatic diazonium ions couple with active substrates such as amines and phenols (Schirmer, 1982; Norwitz and Keliher, 1981and Baiocchi et al., 1982). Because of the size of the attacking species, substitution is mostly para to the activating group. Unless that position is already occupied, ortho substitution takes place. In case of cardol, the substituent being in the meta position, the substitution is preferably in the para position.pH of the media is of paramount importance for the activation of the substrates. For phenols, alkaline medium is recommended because phenols themselves are not active enough for the diazotization reaction. Nevertheless, there is a risk of unstable derivatives and large values of blank due to the process called hydroxy-de-diazonization (March, 1992) which reacts with excess of the reagent in basic medium. 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 cardol in sodium hydroxide medium to produce orange yellow color dye.

The methods involve the coupling of the diazotized sulfa drug with cardol to produce an orange yellow colored product with maximum absorption at 440 nm. The $NH_2$ group of the sulfa drug gets readily diazotized during the diazotisation process to produce diazonium group which reacts with cardol in sodium hydroxide medium to produce an orange yellow colored dye. The reproducibility, sensitivity and adherence to Beer's law with respect to color development were investigated for each reagent separately. 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%.

**Spectral characteristics:**

An orange yellow colored product with maximum absorption at 440 nm was formed when sulfanilamide, sulfadoxine or sulfamethoxazole reacted with cardol in sodium hydroxide medium.

**Optimization of analytical variables:**

In the case of cardol, sodium nitrite (1.0% w/v for SAA and 0.5% 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 gave reproducible results. Hence, sodium nitrite, sulphamic acid and sodium hydroxide each at 1 ml were found appropriate. Experiments were also carried out to find out the amount of SAA, SDX and SMX. It was found that 1–2 ml of 0.25% (w/v) SAA, SDX and SMX gave maximum color intensity. Hence, 1 ml of SAA, SDX and SMX were found appropriate.

**Analytical data:**

A linear correlation was found between absorbance at $\lambda_{max}$ and concentration of all drugs in the ranges given in Table 1. Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope, intercept and correlation coefficient for each system and the values are presented in Table 1. The optical characteristics such as Beer's law limits and Sandel's sensitivity values for all the three methods are given in Table 1.

**Precision and Accuracy:**

Intra-day precision was assessed from the results of six replicate analyses. The mean values and relative standard deviation (RSD) values for replicate analyses at three different levels (amounts/concentrations) were calculated. To evaluate the inter-day precision, analysis was performed over a period of five days, preparing all solutions afresh each day.

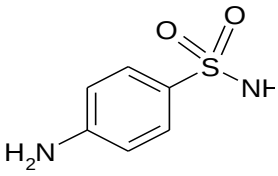
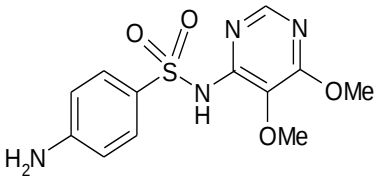
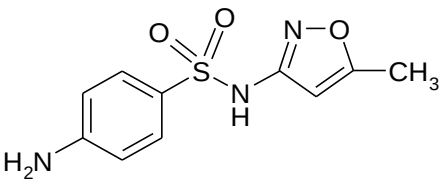
**Robustness and Ruggedness:**

Robustness of the proposed methods was carried out by usual procedure. To check the ruggedness, analysis was performed by three different analysts and on three different spectrophotometers by the same analyst.

**Interference:**

The effect of various anions and cations on the determination of cardol 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 cardol. The results showed that the methods are free from interference by any of the anions and cations, studied.

**Table 1. Optical characteristics for the determination of cardol**

| Parameters |  |  |  |
|---|---|---|---|
| | **SAA** | **SDX** | **SMX** |
| **Colour** | **Orange yellow** | **Orange yellow** | **Orange yellow** |
| **λmax (nm)** | **440** | **440** | **440** |
| **Stability (h)** | **6** | **6** | **6** |
| **Beer's law range (µg $ml^{-1}$)** | **1.0-3.2** | **0.4-5.6** | **0.4-6.4** |
| **Recommended concentration (µg $ml^{-1}$)** | **2** | **2.4** | **3.2** |
| **Molar absorptivity (L $mol^{-1}cm^{-1}$)** | **4.52 x 104** | **4.99 x 104** | **5.39 x 104** |
| **Sandel's sensitivity (µg $cm^{-2}$)** | **0.00567** | **0.00320** | **0.00940** |
| **Regression equation*** | | | |
| **Slope (a)** | **0.183** | **0.130** | **0.137** |
| **Intercept (b)** | **-0.077** | **0.048** | **-0.111** |
| **Correlation coefficient** | **1.00** | **1.00** | **0.988** |

*y= ax+b where x is the concentration of cardol in µg $ml^{-1}$, SAA: sulfanilamide, SDX: sulfadoxine, SMX:sulfamethaxazole

**Table 2. Effect of anions on the determination of cardol**

| Anion salt added | Quantity added (mg) | % Recovery ± RSD** |
|---|---|---|
| Ammonium phosphate | 100 | 99.2 ± 1.09 |
| Calcium carbonate | 100 | 97.1 ± 0.88 |
| Potassium bromate | 100 | 100.6 ± 0.98 |
| Potassium chloride | 100 | 100.6 ± 1.05 |
| Potassium iodate | 100 | 97.0 ± 0.86 |
| Potassium sulphate | 100 | 99.8 ± 0.98 |
| Sodium fluoride | 100 | 101.4 ± 1.02 |
| Sodium nitrate | 100 | 98.5 ± 0.95 |
| Sodium phosphate | 100 | 99.6 ± 1.03 |
| Sodium sulphate | 100 | 101.1 ± 1.00 |

**relative standard deviation, n=5

**Table 3. Effect of cations on the determination of cardol**

| Cation salt added | Quantity added (mg) | % Recovery ± RSD** |
|---|---|---|
| Barium sulphate | 100 | 99.8 ± 0.90 |
| Cadmium sulphate | 100 | 101.1 ± 0.64 |
| Copper sulphate | 100 | 98.6 ± 1.06 |
| Lead nitrate | 100 | 100.0 ± 0.82 |
| Magnesium sulphate | 100 | 97.6 ± 0.76 |
| Manganese sulphate | 100 | 99.5 ± 0.94 |
| Potassium dichromate | 100 | 98.4 ± 1.02 |
| Strontium nitrate | 100 | 100.8 ± 1.14 |
| Tin chloride | 100 | 99.7 ± 0.88 |
| Zinc sulphate | 100 | 98.6 ± 0.92 |

**relative standard deviation, n=5

## IV. CONCLUSION

The procedures described here in involve the use of sulfa drugs containing amino group as spectrophotometric reagents for the determination of cardol, a phenolic compound found in agriculture by-product - cashew nut shell liquid. 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 reaction of cardol with sulfa drugs. Further, a value addition to this method can be achieved if the procedure is made on-line or at-line system and this possibility are currently under investigation.

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