Assay of Vancomycin and Dobutamine Using Sodium Metaperiodate

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Abstract. Three simple and sensitive visible spectrophotometric methods (A–C) are described for the assay of vancomycin (VCM) and dobutamine (DB) (in pure form and in pharmaceutical formulations). These methods are based on the oxidation of vancomycin or dobutamine with excess sodium metaperiodate. Either the products formed (liberated aldehyde with 3-methyl-2-benzothiazolinone hydrazone (MBTH), method A; iodate with p-methylanilinophenol sulphate (PMA), sulphanilamide, in the presence of Mo (VI), method B) or the amount of periodate consumed (celestine blue in the presence of tellurium (IV), method C) are estimated. In this paper the reaction mechanism is presented and all the variables are optimized. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 2.5–30, 0.6–6.0; 2–24, 0.8–9.6 and 2–24, 0.76–6.0 μg ml⁻¹ for methods A, B and C of VCM and DB respectively. The validity of the proposed methods was tested by analyzing pharmaceutical formulations containing drug (VCM, DB) and the relative standard deviations were within 1.0%. Recoveries are 99.55–99.94%.

Key words: Spectrophotometry; vancomycin; dobutamine; sodium metaperiodate; 3-methyl-2-benzothiazolinone hydrazone; celestine blue.

Vancomycin is a glycopeptide antibiotic. Dobutamine is a direct acting sympathomimetic agent and is employed for its inotropic effect in the treatment of congestive heart failure. VCM is chemically known as (S)-(3S, 6R, 7R, 22R, 23S, 26S, 36R, 38aR)-44-[2-O-(3-amino-2,3,6-trideoxy-3-C-methyl-α-L-lyxo-hexopyranosyl)-β-D-glucopyranosyl]-oxy-3-(carbamoyl methyl)-10,19-dichloro-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-7,22,28,30,32-pentahydroxy-6-[(2R)-4-methyl-2-(methylamino)valeramido]-2,5,24,38,39-penta oxo-22H-8,11,18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1H,16H-[1,6,9]oxadiazacyclopentadecino[4,5-m] - [10, 2, 16] benzoazadicycloptetrasine-26-carboxylic acid. DB is chemically known as (±)-4-[2-(3-(4-hydroxyphenyl)-1-methyl propyl amino) ethyl] pyrocatechol. These drugs are officially recognized in B.P [1] and U.S.P [2]. A survey of literature revealed that few methods based on visible spectrophotometry for VCM [3, 4] and DB [5–11] have been reported. Other methods include HPLC for VCM [12, 13] and DB [14], UV for DB [15], amperometry for VCM [16]. The reported spectrophotometric methods possess deficiencies such as a low λmax value and low sensitivity. Therefore, it is of interest to develop simple and sensitive procedures with higher λmax for the determination of VCM or DB in pure and pharmaceutical formulations.

This paper describes three visible spectrophotometric methods for the determination of VCM or DB by making use of the vicinal diol presence. Sodium metaperiodate is a selective oxidant for compounds with vicinal diols [17] and the reaction results in the rupture of C–C bonds in the vicinal diol bearing unit. In the case of VCM or DB results in the rupture of C–C bonds in vicinal diol bearing unit and resorcinol moiety (VCM) or catechol moiety (DB) to form an aldehyde and its reduced form, iodate, besides its presence unreacted. Sawicki et al. [18] developed a
procedure for determining trace concentration of aldehydes by treating them with MBTH to form an intensely blue coloured coupling product. Sastry et al. [19] suggested a procedure for determining sulphanalidam by treatment with PMAP and iodate to form an intensely purple coloured 2:1 charge-transfer complex between p-N-methyl benzoquinone monoxide (PMBQMI) (formed in situ from PMAP and iodate) and sulphanalidam. Sastry et al. suggested [20] a procedure for determining several drugs by periodate oxidation, the periodate reacted is determined spectrophotometrically in presence of tellurium (IV) by reaction with coloured oxazine dye, celestine blue (CB), 1-amino carboxyl-7-diethylamino-3,4-dihydroxy-5-phenoxazinum chloride (C.I.No. 51050), through decrease in the intensity of the colour. We have applied the above three reagents (NaIO₄/MBTH, NaIO₄/Mo(VI)/PMAP-SA and NaIO₄/Te(IV)/CB for the determination of VCM or DB in pure and pharmaceutical formulations.

**Experimental**

**Apparatus**

A Milton Roy spectronic 1201, UV-visible spectrophotometer with matched quartz cells and a digital pH meter model Elico LI-120 were used for absorbance and pH measurements respectively.

**Reagents and Solutions**

All the chemicals used were of analytical grade and all the solutions were prepared with double distilled water. Freshly prepared solutions were always used.

Aqueous solutions of sodium metaperiodate (BDH, 9.35 × 10⁻³ M), MBTH (Fluka, 8.56 × 10⁻³ M) and acetic acid (Qualigens, 3.5 M) were prepared for method A. Aqueous solutions of sodium metaperiodate (BDH, 9.35 × 10⁻³ M), PMAP (BDH, 8.71 × 10⁻³ M), sulphanalidam (Serva, 2.32 × 10⁻² M), sodium molybdate (Aldrich, 4.13 × 10⁻² M) and potassium acid phthalate buffer (pH 3.0) [21] were prepared for method B. Aqueous solutions of sodium metaperiodate (BDH, 100 μg ml⁻¹), CB (Pharma, 200 μg ml⁻¹), sodium tellurite (BDH, 1 mg ml⁻¹) and hydrochloric acid (E. Merck, 5 M) were prepared for method C.

**Preparation of Standard Drug Solution.** VCM and DB, pharmaceutical grade in powder form, obtained as gift samples from Panacea Biotec Limited, Bombay were used. One mg ml⁻¹ stock solution of drug (VCM or DB) was prepared by dissolving 100 mg of drug in 100 ml of double distilled water. The working standard solutions of VCM (250 μg ml⁻¹ for method A; 200 μg ml⁻¹ for methods B and C) and DB (50 μg ml⁻¹ for methods A and C; 80 μg ml⁻¹ for method B) were prepared by further diluting the stock solution with distilled water.

**Preparation of the Sample Drug Solution.** An amount of sample equivalent to 50 mg of active ingredient (VCM or DB) was transferred into 50 ml volumetric flask, shaken thoroughly with 25 ml of distilled water and subsequently diluted to 50 ml. It was filtered if necessary to obtain clear solution and further diluted as in the standard drug solution preparation.

**Procedures**

**Method A:** Aliquots of standard drug solution [0.5–3.0 ml of VCM (250 μg ml⁻¹)] or DB (50 μg ml⁻¹)], one ml each of NaIO₄ (9.35 × 10⁻³ M) and acetic acid (3.5 M) were delivered into a series of 25 ml calibrated tubes. The total volume in each tube was brought to 10.0 ml with distilled water and the samples were kept in a boiling water bath for 40 min. The solutions were briskly cooled and 1.0 ml (8.56 × 10⁻³ M) of MBTH solution was added. After 15 min, the solutions were diluted by adding distilled water to a total volume of 25 ml. The absorbance was measured at 620 nm against a reagent blank within the stability period (1–50 min). The amount of drug in the sample was computed from the Beer-Lambert plot.

**Method B:** Aliquots of standard drug solution [0.5–3.0 ml of VCM (200 μg ml⁻¹)] or DB (80 μg ml⁻¹)] were taken into a series of 25 ml calibrated tubes. Then 2.0 ml of sodium metaperiodate (9.35 × 10⁻³ M) solution was added to each tube. The total volume in each tube was brought up to 5.0 ml with distilled water and kept in a boiling water bath for 40 min. After cooling to room temperature, 2.0 ml of sodium molybdate (4.13 × 10⁻² M) and 10.0 ml of pH 3.0 buffer solution were added to each tube. After 10 min 1.5 ml of PMAP (8.71 × 10⁻³ M) solution was added. After 2 min, 1 ml of sulphanalidam (2.32 × 10⁻² M) solution was added. The solutions in the calibrated tubes were made up to 25 ml with distilled water. The absorbance was measured at 540 nm against a reagent blank within the stability period (10–50 min). The amount of drug in the sample was computed from the Beer-Lambert plot.

**Method C:** Aliquots of standard drug solution [0.5–3.0 ml of VCM (200 μg ml⁻¹)] or DB (50 μg ml⁻¹)] were transferred into a series of 25 ml calibrated tubes containing 3.0 ml of 5 M HCl and 2.0 ml of sodium metaperiodate (100 μg ml⁻¹). The total volume was brought up to 13 ml with distilled water and kept in a boiling water bath for 40 min. After cooling to room temperature, 2.0 ml Te (IV) (1 mg ml⁻¹) solution and 10.0 ml CB (200 μg ml⁻¹) solution were added successively and the absorbances were measured after 15 min at 540 nm against distilled water. In the same way corresponding blank (without the drug) and dye (without the drug and periodate) solutions were prepared and their absorbances were measured against distilled water. The decrease in absorbance corresponding to the consumed periodate, and in turn to drug content, was obtained by subtracting the decrease in absorbance of the test solution (dye minus test) from that of the blank solution (dye minus blank). The amount of drug in the sample was obtained from the Beer-Lambert plot.

The reaction sequence in the existence of coloured species in methods A–C appears to be as follows through analogy and probability. As VCM and DB contain vicinal diol besides amino and catechol unit respectively, sodium metaperiodate offers a wide scope for analytical determination [17]. All the three methods proposed involve two steps. Reaction between this oxidant and VCM or DB leads to formation of an aldehyde and iodate besides unreacted periodate. The type of products obtained in the step I are common. However different experimental conditions were employed in the methods A, B and C so as to suit the subsequent reaction in step II of each one. Method A permits the determination of the liberated aldehyde directly in the reaction medium colorimetrically by an oxidative coupling reaction with MBTH as already illustrated by Sawicki et al. [18]. In method B, VCM or DB is determined by a method involving oxidation with sodium metaperiodate, masking