# Difference Spectrophotometric Assay of 1,2-Diphenolic Drugs in Pharmaceutical Formulations II: Germanium Dioxide Reagent

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**Abstract** □ A rapid, difference UV spectrophotometric assay of formulated drugs containing a 1,2-diphenolic group is described. In this assay are utilized the bathochromic shift of the absorption band of 1,2-diphenolic substances from ~280 to ~287 nm and the concomitant hyperchromic effect induced by the addition of germanium dioxide to an aqueous solution of the drug buffered at pH 6. The absorbance of the solution of the complexed drug relative to that of an equimolar solution of the free drug, which is maximum at ~292 nm, is proportional to the concentration of the drug and is unaffected by the presence of other UV-absorbing substances in the formulations that lack the 1,2-diphenolic moiety. Applications of the assay are described for formulations containing epinephrine, isoetharine, isoproterenol, levodopa, and methyldopa.

**Keyphrases** □ 1,2-Diphenolic drugs—difference UV assay in formulations □ Difference absorption spectrophotometry—assay of 1,2-diphenolic substances

To avoid the interference that may occur in conventional UV-spectrophotometric procedures for the determination of 1,2-diphenolic drugs [*e.g.*, epinephrine (adrenaline), levodopa, and methyldopa] in formulations due to the presence of coformulated drugs, oxidation products, and formulation excipients, many of the absorption spectrophotometric methods currently employed involve the conversion of the drugs to colored derivatives and the measurement of absorbance in the visible region (1–5). In a previous report (6), a new difference UV-spectrophotometric procedure for drugs containing a 1,2-diphenolic moiety was described, based on the measurement of the difference absorbance ($\Delta A$) at ~292 nm between two equimolar solutions of the drug in pH 7 phosphate buffer, one of which also contains 0.1 M boric acid. The boric acid esters of the drugs form very rapidly and have a longer wavelength of maximum absorption ($\lambda_{max}$) and a greater absorptivity than the nonesterified drug. The absorbance of the solution containing boric acid relative to that of the nonesterified drug is proportional to the concentration of the drug and is unaffected by the presence of other UV-absorbing substances in the sample, including monophenols, provided that the absorbance of these substances at the wavelength of measurement is unchanged by the boric acid.

Dihydroxylic and polyhydroxylic substances are known to react with other inorganic acids in addition to boric acid, *e.g.*, arsenious acid (7), orthotelluric acid (8), and germanium dioxide[1] (9, 10). The reaction of germanium dioxide with certain dihydroxylic and polyhydroxylic reagents has been used in both the identification (11) and quantitative determination (12) of trace levels of germanium. In this report, the reagent germanium dioxide is examined for application in the difference spectrophotometric assay of 1,2-diphenolic drugs.

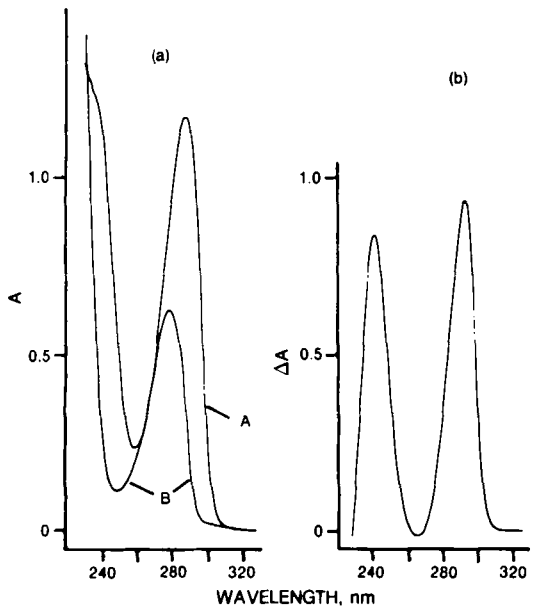


**Figure 1**—*(a) UV absorption spectra of aqueous solutions of epinephrine bitartrate ($2 \times 10^{-4}$ M) at pH 6 in the presence (A) and absence (B) of germanium dioxide (0.4 mg·mL$^{-1}$). (b) Difference UV absorption spectrum of epinephrine bitartrate ($2 \times 10^{-4}$ M) (solution A relative to solution B).*

## EXPERIMENTAL SECTION

**Apparatus**—Absorption and difference absorption spectra of solutions in 1-cm silica quartz cells were recorded from 330 to 220 nm with a recording double-beam UV-visible spectrophotometer[2]. The spectral slit width was 1 nm, the scan speed was 1 nm s$^{-1}$, and the response (time constant) was 0.5 s.

**Reagents**—Epinephrine bitartrate[3], isoproterenol sulfate[4], isoetharine hydrochloride[5], levodopa[6], and methyldopa[6] were obtained commercially. Stock buffer (pH 6) was prepared by dissolving 9.67 g of citric acid and 22.40 g of anhydrous sodium monohydrogen phosphate in water and diluting to 500.0 mL. The working buffer (pH 6) was prepared by diluting 100.0 mL of stock buffer to 500.0 mL with water. The germanium dioxide reagent was prepared by dissolving, with heat, 500 mg of germanium dioxide[3] in 100.0 mL of stock buffer (pH 6); the solution was allowed to cool and diluted to 500.0 mL with water.

**Standard Solutions**—Approximately 50 mg of the reference substance was accurately weighed into a 250.0-mL calibrated flask, dissolved in 50 mL of water and 2.5 mL of 0.1 M HCl[7], and diluted to volume with water. A 5.0-mL aliquot was transferred to each of two 25.0-mL calibrated flasks containing 10 mL of pH 6 buffer and 10 mL of germanium dioxide reagent, respectively, and diluted to volume with water. Five minutes later, the absorbance (at the wavelength of maximum difference absorbance, ~292 nm) of the solution containing germanium dioxide in the sample cell was measured relative to that of the free drug in the reference cell. The measured difference in absorbance was corrected only for the blank buffer solutions, which were prepared by diluting the pH 6 buffer and the germanium dioxide reagent (2:3) with water, as described above for the standard solutions.

**Sample Solutions**—*Aqueous Formulations*—The samples were diluted with water to give a 0.2-mg·mL$^{-1}$ drug concentration based on the declared

[1] Formerly known as germanic acid.
[2] 552 spectrophotometer; Perkin-Elmer Corp.
[3] BDH Chemicals Ltd., Poole, Dorset BH12 4NN, United Kingdom.
[4] Halewood Chemicals Ltd., Staines, Middlesex TW19 6BJ, United Kingdom.
[5] Riker Laboratories, Loughborough, Leicestershire LE11 1EP, United Kingdom.
[6] Merck Sharp & Dohme Research Laboratories, Hoddesdon, Herts. EN11 9BU, United Kingdom.
[7] To prevent oxidation of the drug.



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**Table I—Substances Displaying Difference Absorbance at 292 nm**

| Substance | Phenol Classification | $\lambda_{max}$ of $\Delta A(GeO_2)$, nm | $\Delta\epsilon_{292}$ ($GeO_2$) | $\Delta\epsilon_{292}$ ($GeO_2$)/$\epsilon_{280}$ of Free Drug | $\Delta\epsilon_{292}$ ($H_3BO_3$)/$\epsilon_{280}$ of Free Drug |
|---|---|---|---|---|---|
| Epinephrine | 1,2-Diphenol | 291.5 | 4366 | 1.64 | 0.94 |
| Isoetharine | 1,2-Diphenol | 292.0 | 4523 | 1.48 | 0.89 |
| Isoproterenol | 1,2-Diphenol | 292.0 | 4442 | 1.49 | 0.88 |
| Levodopa | 1,2-Diphenol | 292.5 | 4358 | 1.56 | 0.91 |
| Methyldopa | 1,2-Diphenol | 292.5 | 4306 | 1.40 | 0.85 |
| Carbidopa | 1,2-Diphenol | 292.5 | 4216 | 1.58 | 0.70 |
| Norepinephrine | 1,2-Diphenol | 291.5 | 4211 | 1.62 | 0.91 |
| Dopamine | 1,2-Diphenol | 292.5 | 4077 | 1.43 | 0.82 |
| 1,2-Benzenediol | 1,2-Diphenol | 287.0 | 2629 | — | — |
| 3-Methyl-1,2-benzenediol | 1,2-Diphenol | 285.0 | 1631 | — | — |
| Pyrogallol | 1,2,3-Triphenol | 280.0 | 93 | — | — |
| *n*-Propyl Gallate | 1,2,3-Triphenol | 306.0 | 6115 | — | — |
| Benserazide | 1,2,3-Triphenol | 283.0 | 250 | — | — |

concentration of the drug in the formulation, and the assay was continued as described above.

*Tablets*—Twenty tablets were weighed and powdered. Powder containing ~20 mg of drug was shaken mechanically with 80 mL of water and 1 mL of 0.1 M HCl[7] for 30 min and then diluted with water to 100.0 mL in a calibrated flask. The extract was clarified through filter paper[8]. The first 10 mL was discarded, and the assay was continued as described above.

**Treatment of the Results**—The concentration of drug in the sample solutions, and hence in the sample, was calculated from the proportional relationship that exists between the measured difference absorbance and the concentration of the drug.

## RESULTS AND DISCUSSION

Potentiometric investigations have shown that strong chelates are formed between germanium and dihydroxylic and polyhydroxylic compounds with at least two hydroxyl groups in the vicinal position. Germanium-ligand chelates, 1:1 or 1:2, are formed with glycols and polyhydroxylic sugars (13), whereas 1,2-diphenols form chelates with 1:3 ratios (14).

Complexation of 1,2-diphenols with germanium dioxide, like that with boric acid (6), induces a bathochromic shift of their UV-absorption bands (15). The effect is illustrated in Fig. 1a for aqueous solutions of epinephrine at pH 6, where a shift in the $\lambda_{max}$ from 279 nm to 287 nm and a large increase in absorbance in the presence of germanium dioxide is shown. The maximum $\Delta A$ of the solution of epinephrine in the presence of germanium dioxide relative to an equimolar solution of free drug occurs at 291.5 nm, which is almost identical to that of the epinephrine–boric acid product (6). The magnitude of the $\Delta A_{291.5}$ generated by the germanium dioxide reagent was, however, ~75% greater than that given by boric acid (Table I). Other 1,2-diphenolic substances give similar values for the wavelength of maximum $\Delta A$ (292 ± 0.5 nm) [except for 1,2-benzenediol (287 nm) and 3-methyl-1,2-benzediol (285 nm)] with a corresponding increase in $\Delta A$ values compared with those given by boric acid (Table I).

The pH and the concentration of germanium dioxide were chosen to provide conditions of high sensitivity, reproducible $\Delta A$, and a proportional relationship between the measured $\Delta A$ and concentration of drug. The effects of variation of pH and concentration of germanium dioxide on the absorbance at 291.5 nm of equimolar solutions of epinephrine ($1.4 \times 10^{-4}$ M) are shown in Fig. 2. The highest $A_{291.5}$ was obtained with the higher pH values and concentrations of germanium dioxide. Parallel investigations of the assay conditions, however, showed that, although there was a linear relationship significant at the 99% confidence level between the measured $\Delta A_{291.5}$ and the concentration of epinephrine, a small but not insignificant negative intercept was consistently obtained when the solutions were buffered at pH 7. This was not observed with solutions at pH 6. Therefore, pH 6 and a final germanium dioxide concentration of 0.4 mg·mL$^{-1}$ ($3.8 \times 10^{-3}$ M) were chosen for the assay as a compromise which satisfied the requirements of high sensitivity and the proportionality of the measured value with concentration of analyte. The smaller concentration of germanium dioxide necessary for maximum complex formation compared with that of boric acid (0.1 M) is consistent with the observation that the stability constants of 1,2-diphenol–germanium complexes are considerably higher than those of the boric acid esters (15).

Although the 1,2-diphenol–germanium complexes appear to form rapidly, as observed by an almost instantaneous increase in the $\Delta A_{292}$ on mixing of the reagents, there is a gradual decrease of ~2% in the $A_{292}$ of the complex during the first 5 min, after which the $A_{292}$ and $\Delta A_{292}$ are stable for at least an additional 30 min. Measurement of the $\Delta A$ was, therefore, carried out 5 min after the preparation of the solutions.

**Validation**—All five drugs for which assays of formulations have been developed (Table II) were found to show a proportional relationship between the $\Delta A$ at its $\lambda_{max}$ at ~292 nm and the concentration of the drug in the range of 0–60 μg·mL$^{-1}$ when the solutions were buffered at pH 6. For example, the linear regression equation for epinephrine bitartrate was $y = 0.01307x - 0.0011$, where $y$ is $\Delta A_{291.5}$ and $x$ is the concentration of the drug in micrograms per milliliter ($n = 6$; correlation coefficient = 0.99998). The other four drugs also gave satisfactory linear graphs with intercepts which, being <0.5% of the measured $\Delta A$ at the analytical concentration of 40 μg·mL$^{-1}$, were negligible. For comparison, the linear regression equation for a similar series of solutions of epinephrine bitartrate buffered at pH 7 was $y = 0.01388x - 0.0224$, confirming the slightly greater sensitivity and unsatisfactory proportionality of the measured value and concentration at this pH.

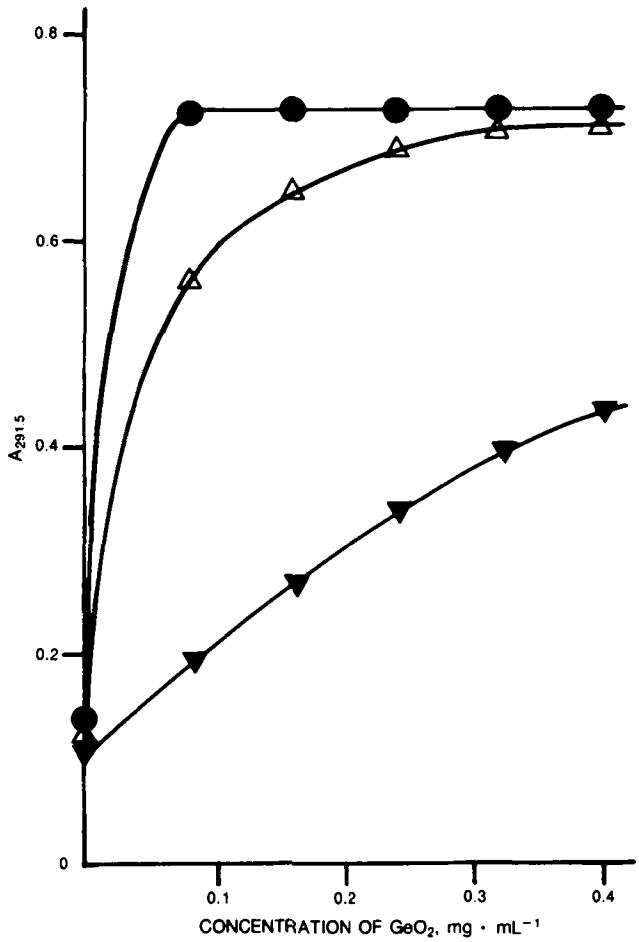


**Figure 2**—*Effects of variation of pH and concentration of germanium dioxide ($GeO_2$) on absorbance at 291.5 nm of epinephrine bitartrate ($1.4 \times 10^{-4}$ M). Key: (●) pH 7; (△) pH 6; (▼) pH 5.*

[8] Whatman No. 1.

**Table II—Assay Results**

| Drug | Formulation | Label Claim Amount | Source[a] | Other UV-Absorbing Components[b] | Amount Found, % of Label Claim | |
|---|---|---|---|---|---|---|
| | | | | | $\Delta A$ Method | Official Method |
| Epinephrine bitartrate | Injection BP | 1.8 mg mL$^{-1}$ | E | — | 99.5 | 98.1[c] |
| | | | C[d] | — | 99.9 | 98.6[c] |
| | Solution BP | 1.8 mg mL$^{-1}$ | E | Chlorocresol, 1 mg mL$^{-1}$ | 101.2 | 100.0[c] |
| | | | C[e] | Chlorocresol, 1 mg mL$^{-1}$ | 97.4 | 97.1[c] |
| | Eye-drops with zinc sulfate BP | 0.9 mg mL$^{-1}$ | E | — | 99.5 | 99.0 |
| | | | C[f] | — | 99.0 | 99.0 |
| | Spray, Co. with atropine BPC | 8.0 mg mL$^{-1}$ | E | Papaverine HCl, 8 mg mL$^{-1}$ | 100.8 | 99.0 |
| | | | C[g] | Papaverine HCl, 8 mg mL$^{-1}$ | 100.8 | 98.7 |
| Isoetharine hydrochloride | Tablet | 10 mg | C[h] | — | 98.1 | — |
| Isoproterenol sulfate | Tablet BP | 10 mg | C[i] | — | 100.9 | 100.5 |
| | Spray BPC (1968) | 10 mg mL$^{-1}$ | E | — | 100.8 | 99.5 |
| | | | C[j] | — | 100.0 | 99.6 |
| Levodopa | Tablet BP | 500 mg | C[k] | — | 98.0 | 99.0 |
| Methyldopa | Tablet BP | 125 mg | C[l] | — | 101.7 | 102.7 |
| | Tablet BP | 250 mg | C[m] | — | 101.0 | 101.8 |
| | Tablet | 250 mg | C[n] | Hydrochlorothiazide, 15 mg | 98.7 | — |

[a] E, prepared sample; C, commercial sample. [b] Compounds absorbing at $\lambda_{max}$ of free drug at ~280 nm. [c] Gravimetric stage only; for discussion refer to Ref. 6. [d] Lot 2EP082, Evans. [e] Lot 7H046, Evans. [f] Lot R8208, Macarthys. [g] Lot 4B028, Evans. [h] Lot 2B01G, Riker. [i] Lot 032124C, Charnwood Pharmaceuticals. [j] Lot 98174, Thornton and Ross. [k] Lot 8056, Brocades. [l] Lot 6770B022, Norton. [m] Lot 2432, Cox. [n] Lot 22034, Merck Sharp and Dohme.

In precision studies in which a sample of adrenaline injection BP was assayed 10 times by the described procedure, the mean concentration was found to be 100.7% of the declared amount, and the *RSD* of $\Delta A_{291.5}$ of the sample solutions was 0.27%, indicating that the reproducibility of the measured value was excellent.

To investigate the specificity of the procedure for 1,2-diphenolic substances, a number of monophenols, diphenols, and triphenols were examined for difference absorbance at 292 nm under the conditions of the assay. The results in Table I indicate the $\Delta\epsilon_{292}$ for those substances that contain a 1,2-diphenolic group. Also shown are the ratios of difference absorptivity at 292 nm, using germanium dioxide and boric acid reagents, to the absorptivity of the free drug at its $\lambda_{max}$ (~280 nm). The results demonstrate that the $\Delta A$ generated by germanium dioxide is 40–64% greater than the absorbance of the free drug and that the $\Delta A$ procedure using germanium dioxide is more sensitive than both a conventional UV-spectrophotometric assay and the $\Delta A$ procedure using boric acid.

Substances containing a 1,2,3-triphenolic group show difference absorbance, also observed for the boric acid reagent (6), although the $\lambda_{max}$ of the complexes and their $\Delta\epsilon_{292}$ are much less consistent than those given by the 1,2-diphenols.

Monophenols (*o*-, *m*-, and *p*-cresols; *o*-, *m*-, and *p*-aminophenols; 4-methyl-2-nitrophenol; 2-methoxyphenol; 4-chloro-*m*-cresol; *p*-hydroxybenzoic acid; orciprenaline; acetaminophen; 2,7-dihydroxynaphthalene; salicylaldehyde), diphenols (resorcinol and hydroquinone), and a triphenol (phloroglucinol) lacking the 1,2-diphenolic group do not display difference absorbance and do not interfere in the $\Delta A$ procedure for 1,2-diphenolic substances. Salicylic acid and salicylamide, which were found to give weak $\Delta A_{292}$ values with boric acid (6), gave zero $\Delta A_{292}$ values with the germanium dioxide reagent. Papaverine, hydrochlorothiazide, and atropine, which are coformulated drugs in certain formulations of 1,2-diphenolic drugs (Table II), also give zero $\Delta A_{292}$ values and do not interfere in the assay. The coincidence of the isosbestic points at ~270 and 263 nm in the difference spectra of the sample solutions and appropriate reference solutions (*i.e.*, wavelengths of zero difference absorbance due to the equal absorptivity of the complexed and free forms of the drug) provide additional evidence of the selectivity of the procedure (6).

**Assay Results**—The accuracy of the $\Delta A$ procedures for formulations was investigated by comparing the results obtained in this study with those given by the official procedures of the British Pharmacopoeia (16, 17) and British Pharmaceutical Codex (18, 19) for preparations containing a 1,2-diphenolic drug, that were either commercial samples purchased locally or samples prepared in the laboratory by compendial formulations.

The results in Table II indicate that good agreement was achieved between the assay values of the $\Delta A$ procedure and the official procedures for both the prepared samples and the commercial samples, including those containing UV-absorbing substances that interfere in a conventional UV assay. The excellent recoveries of added drugs in the prepared samples confirm the accuracy of the $\Delta A$ method and indicate its application as a rapid and selective procedure for the assay of 1,2-diphenolic drugs in formulations.

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