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# Assay of Benzenoid Drugs in Tablet and Capsule Formulations by Second-Derivative Ultraviolet Spectrophotometry


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**Abstract** □ Rapid second-derivative assay procedures are described for tablet and capsule formulations of benzenoid drugs, which eliminate the interference of the formulation excipients in simple UV spectrophotometric methods. Accuracy, precision, and selectivity of the technique are discussed. The general application of the procedures for the batch assay of tablet and capsule dosage forms containing ≥2 mg of a benzenoid drug is indicated by the results obtained for 18 such formulations, in good agreement with pharmacopeial assay results and/or declared amounts. The procedures are sufficiently sensitive to permit unit dose assays of these formulations.



**Keyphrases** □ Benzenoid drugs—tablet and capsule formulations, second-derivative UV spectrometry □ Formulations—benzenoid drugs, second-derivative UV spectrometry, tablets and capsules □ UV spectrometry, second-derivative—benzenoid drugs, tablet and capsule formulations


The interference of formulation excipients in the conventional ultraviolet (UV) spectrophotometric procedures for certain formulated drugs is well recognized. It is a particular problem in the assay of tablets and capsules of benzenoid drugs, which, in general, are both weakly absorbing and also formulated at a relatively low dosage level (typically 1–50 mg/unit dose). The high excipient–drug ratio and high sample weight required for these formulations result in background-irrelevant absorption of a sufficiently high intensity to possibly prohibit the application of simple spectrophotometric methods.

Techniques that reduce or eliminate matrix interference in the assay of formulated drugs include the Morton–Stubbs (1) correction procedure, which requires that the irrelevant absorption is linear over the wavelength range of the absorption band of the drug; orthogonal polynomials, which can cope with nonlinear irrelevant absorption if the correct choice of the polynomial order, wavelength range, and interval is made (2, 3); compensation spectrophotometry, in which the reference solution contains the matrix or sample at the same concentration present in the sample solution (4, 5); and difference spectrophotometry, which may be applied if an absorbance difference can be


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**Table I—Assay Results**

| Drug | Analytical Concentration, mg/mL | Measured[a] Amplitude | Specific[b] Amplitude | Formulation[c] | Stated Amount per Unit, mg | Found, % of Declared Amount: Derivative Method | BP[d] Method | Uncorrected Absorbance |
|---|---|---|---|---|---|---|---|---|
| Trihexiphenidyl hydrochloride | 0.6 | $257_S$ | 0.86 | T | 2 | 97.4 | 95.7 | 179.9 |
| Benztropine mesylate | 0.65 | $258_S$ | 0.80 | T | 2 | 100.6 | 98.7 | 167.2 |
| Bethanidine sulfate | 0.5 | $257_S$ | 1.06 | T | 10 | 99.8 | 99.2 | 136.2 |
| Bretylium tosylate | 0.2 | $278_S$ | 2.51 | T | 50 | 101.4 | NA | 115.6 |
| Chlorcyclizine hydrochloride | 0.25 | $268_L$ | 2.26 | T | 50 | 96.7 | 97.3 | 106.0 |
| Cyclizine hydrochloride | 0.11 | $270_S$ | 4.78 | T | 50 | 102.5 | 99.6 | 107.0 |
| Debrisoquin sulfate | 0.25 | $268_L$ | 2.21 | T | 20 | 99.8 | 101.4 | 105.2 |
| Diphenhydramine hydrochloride | 0.5 | $258_S$ | 1.20 | C | 25 | 96.2 | 95.4 | 97.9 |
| Diphenylpyraline hydrochloride | 0.5 | $257_S$ | 1.14 | C | 5 | 102.0 | NA | 124.2 |
| Hydroxyzine hydrochloride | 0.3 | $270_L$ | 1.70 | T | 10 | 96.4 | NA | 139.5 |
| Scopolamine butylbromide | 0.8 | $257_S$ | 0.66 | T | 10 | 97.7 | 95.1 | 129.3 |
| Meclizine hydrochloride | 0.25 | $272_L$ | 2.35 | T | 25 | 101.8 | 103.2 | 106.5 |
| Methadone hydrochloride | 0.65 | $260_S$ | 0.81 | T | 5 | 101.4 | 100.2 | 121.1 |
| Orphenadrine hydrochloride | 0.40 | $270_L$ | 1.51 | T | 50 | 100.2 | 99.9 | 106.8 |
| Oxyphenonium bromide | 0.8 | $257_S$ | 0.64 | T | 5 | 97.2 | NA | 111.2 |
| Meperidine hydrochloride | 0.5 | $257_S$ | 1.04 | T | 25 | 96.9 | 97.0 | 102.3 |
| Phenelzine sulfate | 0.5 | $258_S$ | 1.01 | T | 25.8 | 99.1 | 100.5 | 226.0 |
| Tolazoline hydrochloride | 0.35 | $256_S$ | 1.63 | T | 25 | 97.1 | 98.5 | 100.2 |

[a] Wavelength of minimum (nanometers) measured to the shorter (S) or longer (L) satellite. [b] See text for definition. [c] T = tablet, C = capsule. [d] NA = not applicable.

induced between equimolar solutions of the drug by the addition of reagents to one or both of the solutions, provided the matrix absorbance is unaffected by the reagents (6). The accuracy and specificity of UV absorption methods may also be considerably improved by conversion of the normal zero-order spectrum into a higher order (usually second or fourth) derivative spectrum. The improved resolution of overlapping absorption bands and the discrimination in favor of narrow bands against broader bands, which are the principal characteristics of derivative spectrophotometry (7, 8), can result in the complete elimination of both nonspecific matrix interference (8, 9) and specific interference from coformulated compounds (10–12).

The benzenoid drugs are ideal candidates for derivative measurements, as the narrow absorption bands of the fine structure in the 250–270-nm region give derivative bands of greater amplitudes than many of the more strongly absorbing drugs, which have a wider spectral band width. Several applications of derivative spectrophotometry in the assay of individual benzenoid compounds in formulations have been published (11–13). The purpose of this paper is to report the general application of the technique to a much wider range of these drugs in tablet and capsule formulations.

## EXPERIMENTAL

**Drug Substances**—Scopolamine butylbromide[1], orphenadrine hydrochloride[2], bethanidine sulfate[3], bretylium tosylate[3], chlorcyclizine hydrochloride[3], cyclizine hydrochloride[3], methadone hydrochloride[3], trihexiphenidyl hydrochloride[4], benztropine mesylate[5], debrisoquin sulfate[6], meperidine hydrochloride[6], diphenhydramine hydrochloride[7], diphenylpyraline hydrochloride[8], meclizine hydrochloride[9], hydroxyzine hydrochloride[10], oxyphenonium bromide[11], tolazoline hydrochloride[11], and phenelzine sulfate[12] were used.

[1] Sigma London Chemical Co. Ltd., Poole, Dorset, U.K.
[2] Aldrich Chemical Co. Inc., Gillingham, Dorset, U.K.
[3] The Wellcome Foundation Ltd., Crewe, Cheshire, U.K.
[4] Lederle Laboratories, Gosport, Hants., U.K.
[5] Merck Sharp and Dohme, Ltd., Hoddesdon, Herts., U.K.
[6] Roche Products Ltd., Welwyn Garden City, Herts., U.K.
[7] Park Davis and Co., Pontypool, Gwent, U.K.
[8] Smith Kline and French Laboratories Ltd., Welwyn Garden City, Herts., U.K.
[9] BDH Pharmaceuticals Ltd., Alton, Hants., U.K.
[10] Pfizer Ltd., Sandwich, Kent, U.K.
[11] Ciba Laboratories, Horsham, West Sussex, U.K.
[12] Charles R. Warner and Co. Ltd., Pontypool, Gwent, U.K.

**Spectrophotometry**—Second-derivative absorption spectra of standard and sample solutions, equilibrated to room temperature were recorded in 1-cm silica quartz cells using a double-beam UV–visible recording spectrophotometer[13]. The scan speed was 60 nm/min, the spectral slit width 1 nm, response (time constant) 0.5 s, and the maximum and minimum ordinate settings +0.5 and −0.5, respectively. The unit dose assays of trihexiphenidyl hydrochloride, benzotropine mesylate, methadone hydrochloride, and oxyphenonium bromide required more sensitive ordinate settings of ±0.15, ±0.15, ±0.4, and ±0.3 respectively.

*Standard Solutions*—The second-derivative absorption spectrum was recorded from 290 to 230 nm for a standard solution of the drug, accurately prepared at the analytical concentration specified in Table I. For the unit dose assays of trihexiphenidyl hydrochloride, benztropine mesylate, methadone hydrochloride, and oxyphenonium bromide, standard solutions of 0.2, 0.2, 0.5, and 0.5 mg/mL, respectively, were prepared.

*Sample Solutions*—Twenty tablets or the contents of 20 capsules were weighed and powdered. A quantity of powder which gave an extract of the drug at the concentration, based on the declared strength of the formulation, that is specified for the drug in Table I was weighed into a 50-mL volumetric flask. The powder was shaken with 0.1 M HCl (40 mL) for 20 min and diluted to 50.0 mL with 0.1 M HCl. The extract was filtered through filter paper[14], the first 10 mL of filtrate was discarded to avoid problems arising from adsorption of the drug to the filter, and the second-derivative absorption spectrum of the filtrate was recorded.

**For unit dose assays,** a tablet or the contents of a capsule were powdered in a beaker with a glass rod. A suitable volume of 0.1 M HCl, 10.0–500.0 mL (given by declared unit dose in mg/analytical concentration in mg/mL), was added which yielded an extract containing the specified analytical concentration of the drug. Small volume extracts, <20 mL, were filtered through a membrane filter[15] and larger volume extracts through filter paper[14], as described above, and the second-derivative spectrum of the filtrate was recorded. For the unit dose assays of trihexiphenidyl hydrochloride, benztropine mesylate, methadone hydrochloride, and oxyphenonium bromide, 10.0 mL of 0.1 M HCl was used.

## RESULTS AND DISCUSSION

**Experimental Parameters**—The zero-order and second-derivative absorption spectra of meperidine hydrochloride (pethidine hydrochloride), as an example of a benzenoid drug displaying characteristic fine structure in the region of 250—270 nm, are shown in Fig. 1A and B, respectively. The wavelengths of the minima of the inverted bands (a, b, c, and d) in the second-derivative spectrum correspond, after correction for displacement of the spectrum in the direction of the scan (14, 15), with the $\lambda_{max}$ value (a, b, and c) at 251, 257, and 262.5 nm and shoulder at 268 nm in the zero-order spectrum. The second-derivative spectra of the other

[13] Perkin-Elmer 552 Spectrophotometer, Perkin-Elmer Corp., Norwalk, Conn.
[14] Whatman No. 1.
[15] Millipore Filter, 0.45 μm, 25-mm dia.

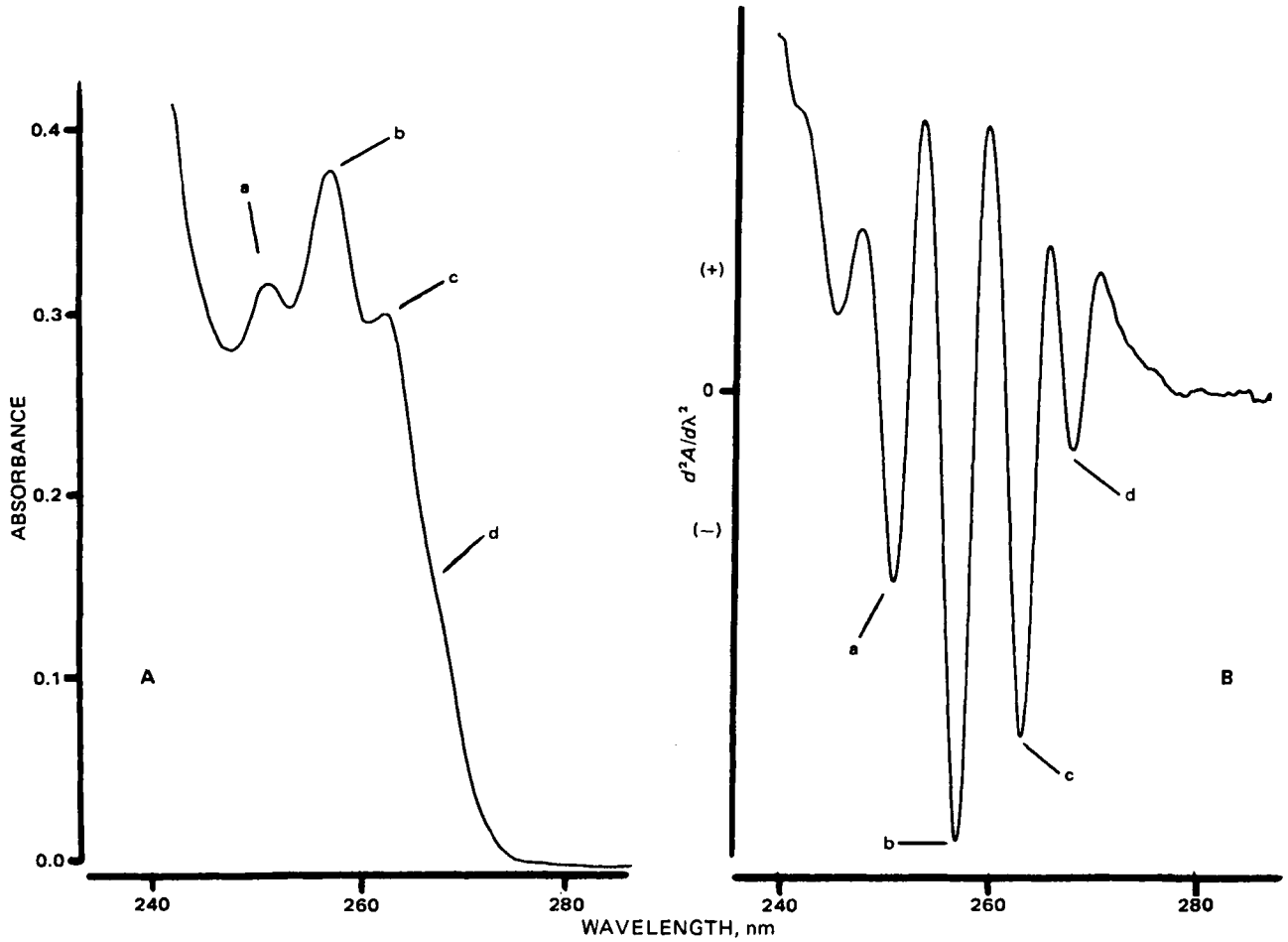


**Figure 1**—*Zero-order (A) and second-order derivative (B) spectra of meperidine hydrochloride (0.5 mg/mL in 0.1 M HCl).*

benzenoid drugs are similar to that of meperidine, differing only in the relative amplitudes of the vibrational bands and the wavelengths at which they occur. The amplitude of the largest band in the derivative spectrum of each drug (*e.g.*, for meperidine at 257 nm measured to its shorter wavelength satellite), providing the greatest sensitivity, was chosen for the analytical measurement.

The solvent used throughout was 0.1 M HCl, which is a satisfactory extracting solvent for these basic drugs, maintaining them substantially in the protonated form. It has been observed during the present work that certain drugs which show a barely discernible difference between the protonated and nonprotonated forms in the zero-order spectra display considerably larger differences in the derivative spectra. For example, the absorbance at $\lambda_{max}$ 262.5 nm of meperidine is only 2.6% greater in 0.1 M HCl than in 0.1 M NaOH, whereas the amplitude of the second derivative minimum at 262.5 nm measured to its longer wavelength satellite is 26.3% greater in 0.1 M HCl than in 0.1 M NaOH.

The instrumental variables (absorbance range, spectral band pass, time constant, scan speed, and recorder voltage) were held constant for all the drugs, and the analytical concentration of each drug was selected as that giving the largest derivative amplitude at 50–60% of the chart full range. This approach enabled a direct comparison of the derivative responses at a constant instrumental damping and noise level to be made. Table I lists the analytical concentration together with the specific amplitude of the largest band, calculated as the amplitude, in millimeters, of a 1-mg/mL solution of the drug at 20°C as a ratio of the full chart width (250 mm) of the recorder on the 1-V range, and given by:

$$\text{Specific amplitude} = \frac{\text{Amplitude (mm)}}{\text{Concentration of drug (mg/mL)} \times 250}$$

**Validation**—The proportionality of the measured amplitude and concentration was checked for each drug by means of a six-point calibration graph at concentrations of 0, 25, 50, 75, 100, and 125% of the analytical concentration. In all cases, a proportional relationship was found to exist, with correlation coefficients ≥0.9996 and intercepts ≤ ±1% of the analytical concentration.

The precision of the amplitude measurements under the instrumental conditions was determined by recording the second-derivative spectra of five drug solutions, at their analytical concentrations, 10 times. The replicate measurements were each made after refilling the cell with fresh solution at room temperature to avoid an increase in the temperature of the solution in the cell compartment, which may reduce the measured amplitude (16). The relative standard deviations of the amplitudes of bethanidine, diphenhydramine, hydroxyzine, cyclizine, and diphenylpyraline all fell in the range of 0.42–0.59%. The similarity of these values is undoubtedly due to the similarity of the measured amplitudes (50–60% of the recorder scale) achieved by the selection of the analytical concentration.

As instrumental noise is a major factor affecting the reproducibility of spectra, the constant instrumental parameters adopted throughout this work would be expected to give constant noise levels, similar signal-to-noise ratios, and simllar satisfactory precision for the other drugs. Furthermore, the sampling procedure for most of the drug formulations may be readily modified to cope with unit dose assays using the same instrumental parameters without loss of precision of the measurement.

Unit dose assays may be carried out by crushing the formulation unit and extracting the drug with a suitable volume of 0.1 M HCl, as little as 10.0 mL if necessary, to obtain the desired analytical concentration, followed by filtration of the extract through a membrane filter or filter paper. It is considered that the systematic error incurred in this procedure by the increase in volume due to the dissolved components of the formulation is negligible.

The only formulations for which the normal instrumental parameters had to be modified for the unit dose assays were of those drugs having small specific amplitude (<1.0) and which are formulated in low dosage (≤5 mg/unit dose), *viz.*, trihexiphenidyl hydrochloride, benztropine mesylate, methadone hydrochloride, and oxyphenonium bromide. The unit dose assay of these formulations required the extraction of a dose unit with 10.0 mL of 0.1 M HCl, membrane filtration, and an increase in the sensitivity of the ordinate scales by factors of 3.33, 3.33, 1.25, and 1.67, respectively, of that of the normal scale. The relative standard deviations of the measurements for those drugs for which the unit dose assay re-

quired the ordinate scale expansion were 0.96, 0.91, 0.62, and 0.58% for trihexiphenidyl hydrochloride, benztropine mesylate, methadone hydrochloride, and oxyphenonium bromide, respectively.

**Assay Results**—Table I records the results obtained for 18 solid dosage formulations of benzenoid drugs which, with the exception of four, are official in the British Pharmacopoeia 1980 (17). The analytical concentration for each drug is given, and the measured amplitude is specified by the wavelength of the minimum in the derivative spectrum, after correction for scan speed effects (14, 15), measured either to its shorter or longer wavelength satellite. The specific amplitude is included to indicate the relative derivative responses of the drugs on a weight basis.

The presence of irrelevant absorption in all the sample extracts was confirmed by the observation of nonspecific absorption above 285 nm (where the drugs, except methadone, have zero absorptivity) and of the increasing distortion of the spectra toward lower wavelengths. The extent of the interference in the sample extracts is evident from the results (in the last column of Table I) showing the assay results calculated from the absorbance of the extracts at the $\lambda_{max}$ in the region of 255–270 nm, uncorrected for irrelevant absorption. The background absorption at the $\lambda_{max}$ of one drug formulation, that of phenelzine sulfate, exceeded the absorbance of the drug itself and many formulations showed interference >10% of that of the drug. The derivative spectra of the sample extracts, however, showed no apparent distortion and were identical in shape to the corresponding standard solutions, indicating the elimination of the broad-band irrelevant absorption of the formulation excipients from the derivative spectra of the sample solutions. In the case of two benzenoid drugs, atropine sulfate and scopolamine hydrobromide, formulated at very low doses (0.3 and 0.6 mg/tablet), there was unacceptable distortion of the second- and even of the fourth-derivative spectra by the tablet excipients. The derivative spectrophotometric assay of these very low-dose formulations by an alternative procedure is the subject of another report (18).

The assay results for the formulations, obtained by the derivative procedure, are in excellent agreement with the declared amount and, where appropriate, with the results given by official procedures of the BP 1980 (17), confirming that second-derivative spectrophotometry is a simple, rapid, and selective technique which has general application in the assay of tablet and capsule formulations containing a single benzenoid drug. The specificity of the procedures for drugs in the presence of degradation products has not been investigated in the present study. It is likely, however, that degradation products will contribute to the derivative spectrum of the sample solution and therefore interfere in the assay, if their zero-order UV spectra, like that of the parent drug, show fine structure in the 250–270-nm region.

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# Estrogenic and Antiestrogenic Activity of Novel Selenosteroids


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Received November 22, 1982, from the **Faculty of Medicine, Kuwait University, Kuwait*, and the ‡ *College of Pharmacy, The University of Oklahoma, Oklahoma City Campus Health Science Center, Oklahoma City, OK 73190.* Accepted for publication February 8, 1983.



**Abstract** □ An assay for estrogenic and antiestrogenic activity of seven selenoestrogens has been carried out in immature female rats. The estrogenic activity was compared to the *in vitro* binding affinity data. The study reveals that introduction of selenium substituents on C-16 or C-17 of the steroid nucleus produced a marked reduction in the estrogenic activity. The selenium analogues of ethynylestradiol produced the highest estrogenic activity. None of the compounds produced antiestrogenic activity.



**Keyphrases** □ Selenoestrogens—estrogenic and antiestrogenic activity in immature rats, comparison with *in vitro* binding affinity □ Estrogens—selenium substituted, estrogenic and antiestrogenic activity in immature rats, comparison with *in vitro* binding affinity □ Binding affinity, *in vitro*—selenium-substituted estrogens, correlation with *in vivo* estrogenic and antiestrogenic activity, rats


The differentiation between estrogen receptor-negative and receptor-positive breast tumors and their metastases is important in the management and treatment of such tumors (1, 2). Present techniques are based on the *in vitro* measurement of estrogen receptors in freshly obtained biopsies of breast tissue. Therefore, a radiopharmaceutical which could be used to image estrogen-dependent breast tumors and metastatic foci by external detection could be a useful diagnostic tool. A number of investigators have attempted to develop such a radiopharmaceutical using radiohalogenated (*e.g.*, iodine-125, bromine-77) estrogens (3, 4). However, most of these studies have met with limited success. Although selenium-75 may not be an ideal radionuclide for diagnostic use, it offers the following advantages (5, 6): (*a*) its long half-life (120 d) allows enough time for synthesis and handling; (*b*) it can be incorporated into organic molecules with minimal difficulty; (*c*) the organoselenium compounds are more stable *in vivo* than the corresponding halogenated derivatives; and (*d*) preliminary studies with selenium-75 could determine the feasibility of using the potentially more useful $^{73}Se$-labeled compounds ($t_{1/2}$ = 7 h).




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