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Application of conventional (DC) and differential pulse polarography (DPP) to the quality control of benzothiadiazines in tablets. Simultaneous determination of hydrochlorothiazide and dihydralazine sulphate in mixtures with minimization of interaction by the use of a zero covariance in the calibration

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A previous study of the polarographic behaviour of 14 benzothiadiazines having diuretic properties enabled possible reaction mechanisms to be proposed and gave the opportunity to determine the best conditions for analytical application. The results obtained with benzthiazide as an example of the unsaturated compounds, bendrofluazide as the dihydro compound, and hydrochlorothiazide determined together with dihydralazine are reported. The electroactivity of these last two substances was made the object of a mathematical study for the correction of reciprocal interference.

Several classical methods have been proposed for the analysis of halogenated derivatives of 1,2,4 benzothiadiazine-1,1 dioxides and of their 3,4-dihydroderivatives. Titrimetry in non-aqueous media described by Kala (1965) is also the official method adopted by the French Pharmacopoeia (1965), the United States Pharmacopeia (1980) and the British Pharmacopoeia (1980). For tablets, u.v. spectrophotometry is preferred by the British Pharmacopoeia. De Paulis & Dipietromaria (1960) describe the analytical properties of chlorothiazide and its photometric determination. Baruffini & Tiengo (1960), Kala (1965) and Sheppard et al (1960) propose the colorimetric determination of the amines liberated by alkaline hydrolysis of these compounds.

The application of polarography to the quantitative analysis of some of the group has also been described: polythiazide (Gantes & Barat 1967), chlorothiazide, dihydrochlorothiazide and methylchlorothiazide in tablets (Khkolos & Walker 1975). The same method had already been used by Francis Summa (1962) and Gantes & Barat (1967) for chlorothiazide and was adopted by the U.S.P. (1975).

After having made a preliminary study of the polarographic behaviour of two unsaturated and 12 saturated compounds which led to the postulation of possible reaction mechanisms (Van Kerchove & Schoenmakers 1982), we describe here the best conditions for the analysis in tablets of benzthiazide and bendrofluazide taken as examples of the two groups. In addition we have compared the accuracy obtained in DPP by two different ways of establishing the calibration curves for tablets containing a mixture of hydrochlorothiazide and dihydralazine sulphate, both reduced at potentials differing by 200 mV.

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MATERIALS AND METHODS

Chemicals

Bendrofluazide was supplied by the Boots Company and by Glaxo-Allenburys. Benzthiazide came from Pfizer Corporation, hydrochlorothiazide and dihydralazine sulphate from Ciba-Geigy. The tablets were commercial samples. Reagents p.a. from Merck and dimethylformamide, quality Uvasol, were used as purchased. Michaëlis phosphate buffer pH 7·3 and borate buffer pH 9·7 (Brezina & Zuman 1958). Borate buffer pH 8·10 was made according to Cohen et al (1962). Commercial buffers were used to calibrate the pH meter Radiometer type 25. Aqueous solutions were made using double-distilled water.

Apparatus

Direct current (DC) and differential pulse polarography (DPP) were performed with a Tacussel PRG 5 type polarographic analyser in conjunction with a

three electrode cell system, the counter electrode being platinum and the reference a saturated calomel electrode. The dropping mercury electrode used was a Tacussel type CMT 5/24 with an outflow velocity of 0·6964 mg/sec at −1·50 V and a mercury pressure of 60 cm in M NaOH. The measuring cell was a shielded cell type CPR 5 B.

Method

All derivatives were measured in buffered solutions, dimethylformamide was used as solvent in two cases. The solutions were degassed with oxygen free nitrogen for 10 min before the polarogram was recorded. The temperature of the cell was thermostatistically controlled at 25 °C.

The conditions normally employed in the DC and DPP investigations were: drop time: 1 s; scan rate 4 mV s^{-1}, modulation amplitude: 50 mV. The records were produced with a potential range of 50 mV cm^{-1}. The calibration curves used were obtained from individual test solutions, after dilution in 20 ml volumetric flasks of the appropriate volumes of freshly-prepared stock solutions in dimethylformamide and addition of the buffers. The concentration range investigated was $5 \cdot 10^{-5}$ to $4 \cdot 10^{-4}$ M.

The results were calculated by multivariate linear regression as developed by Kendall & Stuart (1963).

Analytical applications: content uniformity test in tablets

Benzthiazide: recommended procedure

Transfer one tablet (equivalent to 50 mg benzthiazide) to a 50 ml volumetric flask, grind it roughly with a glass rod and add dimethylformamide to the mark.

Shake the flask for 30 min. Filter the solution and transfer 3 ml of the filtrate to a 20 ml volumetric flask. Add 10 ml of a phosphate buffer pH 7·3 and dilute to the mark with water. Mix. The pH of the final solution is 7·8. Transfer the solution to a polarographic cell, deaerate and record the polarogram from −1 to −2 V. Measure the diffusion current at the peak potentials in DPP and determine the amount of benzthiazide from the first one by means of a calibration curve freshly determined for each series of measurements.

Results and discussion

Each of the two reduction waves of benzthiazide was measured in DC and DPP for the standards as well as for the tablets and the accuracies of the results were compared. Four standards have been used in the concentration range between 50 and 250 μg ml^{-1} ($1 \cdot 15$ to $5 \cdot 8 \times 10^{-4}$ M). A greater accuracy was obtained with the first wave. In DC the mean of the recovered values was 99·35% for the first wave and 100·3% for the second, with a relative standard deviation of 3·18% and 4·23%. In DPP the mean of the recovered values was 100·45% for the first peak and 100·52% for the second, with a standard deviation of 2·108% and 3·06% respectively.

The results of ten polarographic assays of tablets, five ground and five not ground, reported in Table 1 show clearly that the tablet should be ground before extraction.

Table 1. Polarographic DC and DPP analysis of benzthiazide in 10 tablets.

A. DC	First wave		Second wave	
	ground	unground	ground	unground
Average value	50·19 mg	49·71 mg	51·7 mg	49·67 mg
Standard deviation	1·358	1·34	1·31	3·54
Relative s.d.	2·70%	2·70%	2·54%	7·13%
B. DPP	**First peak**		**Second peak**	
Average value	49·18 mg	49·76 mg	50·92 mg	50·29 mg
Standard deviation	0·822	3·53	1·62	3·49
Relative s.d.	2·67%	7·10%	3·18%	6·95%

Both methods give acceptable and comparable results, except for the second peak in DPP, where a greater relative standard deviation is obtained. They are within the limits required for content uniformity by the National Formulary (1975).

Bendrofluazide: recommended procedure

Transfer one tablet (equivalent to 2·5 or 5 mg bendrofluazide) to a 10 ml volumetric flask. Grind it roughly with a glass rod to assist its disintegration in solvent and add dimethylformamide to the mark. Shake the flask for 30 min. Filter the solution and transfer 4 ml of the filtrate to a 25 ml volumetric flask. Dilute to the mark with a borate buffer solution pH 8·10. Mix. Transfer the solution to a polarographic cell, deaerate and record the polarogram in the potential range beween −1·30 and −2 V. Measure the diffusion current, preferably with DPP, at the top of the peak (E_p = −1·75 V) and determine the amount of bendroflumethiazide from a calibration curve produced by the same procedure.

Results and discussion

DC and DPP measurements have been carried out to compare the results obtained by both methods, applied to the standards as well as to the tablets. DC results are much less satisfactory than DPP results (Table 2). The difference arises from the inaccuracies of measuring the limiting current at very negative potentials in the DC wave. The results of 10

polarographic assays of tablets containing 5 mg of bendroflumethiazide gave a mean value of 5·35 mg/tablet with a standard deviation of 0·137 and a relative standard deviation of 2·56% by DPP. The mean value was 5·326 mg/tablet by DC with a standard deviation of 0·276 and a relative standard deviation of 5·20%.

Hydrochlorothiazide in a mixture with dihydralazine sulphate

Recommended procedure for the determination of both substances in tablets which also contain reserpine.

Transfer one tablet (equivalent to 10 mg of each electroactive species) to a 50 ml volumetric flask. Grind it roughly and add Michaelis borate buffer pH 9·7. Shake the flask in the dark for 30 min (to prevent alteration of dihydralazine).

Filter the solution and transfer 6 ml of the filtrate to a 20 ml volumetric flask. Dilute to the mark with the buffer and mix. Transfer the solution to a polarographic cell, deaerate and record the differential pulse polarogram in the potential range between −0·6 and −2 V.

Measure the diffusion currents at both peaks and determine the amount of hydrochlorothiazide and dihydralazine from the calibration curves where the currents of both compounds have to be taken into account (see appendix I and II) and that have been established the same day.

To carry out the calibration, transfer 24·50 mg hydrochlorothiazide, accurately weighed, to a 50 ml volumetric flask (A) Transfer 17·50 mg dihydralazine sulphate (100%), accurately weighed, to a second 50 ml volumetric flask (B).

Dissolve the substances in the borate buffer and dilute the solutions to the mark. Add to four 20 ml volumetric flasks:

	(1)	(2)	(3)	(4)
Solution (A)	3·30 ml	5 ml	2·50 ml	4·15 ml
Solution (B)	2 ml	3 ml	4 ml	5 ml

Dilute each flask to the mark with the buffer, mix and proceed as previously described for the tablets. Calculate the calibration curves by multivariate linear regression.

Results and discussion

Two different calibration methods have been compared. In the first case four concentrations of the two substances were taken and the ratio between them was maintained constant. In the second case, which has been adopted in the recommended procedure, the concentration, A and B, of the two compounds in n solutions are distributed in such a way that their covariance defined as

$$C = \frac{\Sigma AB - (\Sigma A \times \Sigma B)/n}{n - 1} \text{ is equal to zero.}$$

This experimental design ensures orthogonality as defined by Davies (1954), which means minimization of the experimental errors. In addition, a code has been used to simplify the calculations. The procedure to follow is detailed in appendix I. Calculations of the calibration curves have been made by means of matrix algebra, as revised by Massart et al (1978) and with the help of a Texas TI programmable 59 device, using the ML-02 programe. Details of the calculation method are given in appendix II. As can be seen from Table 3 A and B, much better results were obtained in the second case.

The relative standard deviation of 3·45% became 0·88% for hydrochlorothiazide and was also better for dihydralazine. A valuable correction for interaction between the peaks is obtained by the use of this procedure. This is why we used it for quality control on 10 tablets. The mean value was 10·82 mg hydrochlorothiazide per tablet with a standard deviation of 0·517 and a relative standard deviation of 4·78%, which meets the requirements of the U.S.P., XX revision (1980) for the content uniformity, carried out on 10 tablets (85·0–115·0%). The mean value obtained for dihydralazine was 9·26 mg/tablet with a standard deviation of 0·525 and a relative standard deviation of 5·66%.

Table 2. Standardization of bendrofluazide.

	DC		DPP	
Theoretical concn (μg ml^{-1})	Current intensity μA	Results %	Current intensity μA	Results %
24·47	0·180	107·31	0·286	101·61
39·15	0·275	97·18	0·420	100·28
73·41	0·567	101·27	0·740	100·32
122·35	0·92	96·52	1·175	98·40
171·29	1·37	101·54	1·66	100·71
Average of results		100·76		100·26
Standard deviation		4·31		1·17
Relative standard deviation		4·28		1·17

CONCLUSION

The results obtained for the quantitative analysis performed under the conditions described for three benzothiadiazine derivatives, were in agreement with the requirements of the pharmacopoeia. There-

Table 3. Standardization of mixtures of hydrochlorothiazide (H) and dihydralazine (D) after correction for interaction by multivariate linear regression calculation.

A. Standardization of both constituents in mixtures with constant concentration ratio of H/D = 1·14 (DPP measurements)

Hydrochlorothiazide			Dihydralazine		
Theor. concn μg ml^{-1}	Curr. intensity (L_I) μA	Recov. %	Theor. concn μg ml^{-1}	Curr. intensity (L_{II}) μA	Recov. %
39·83	0·46	96·39	34·79	1·30	99·18
59·74	0·695	104·59	51·54	1·94	101·91
79·66	0·910	101·20	68·72	2·41	99·13
99·57	1·19	99·18	86·93	2·86	100·28
Average of results		100·34			100·12
S^2		11·91			1·71
S		3·45			1·31
Relative s.d.		3·44			1·31

B. Standardization of both constituents in mixtures with concentration ratios related to a covariance zero

80·75	0·83	101·31	35·03	1·25	100·84
122·35	1·325	99·78	52·55	2·08	99·42
61·17	0·787	99·44	70·065	2·45	99·59
101·55	1·30	100·74	87·58	3·33	100·34
Average of results		100·33			100·05
S^2		0·77			0·44
Relative s.d.		0·88			0·66

fore we consider the proposed working conditions as suitable for quality control in different formulations. In the case of mixtures of two electro-reducible substances we recommend that the concentrations of the respective standards be chosen in such a way that the covariance is zero. We also recommend the use of the multivariate linear regression calculation to eliminate interaction. The conditions described gave the most satisfactory results.

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APPENDIX I: Calibration procedure with a zero covariance.

Consider as an example the conditions described for the simultaneous determination of hydrochlorothiazide (A) and dihydralazine sulphate (B) (case 3 of the analytical applications). The amounts of species A added to a series of regularly increasing concentrations of species (B) are chosen according to a special plan, characterized by the fact that the ratio of both species is not kept constant. To simplify the calculations, a code is used where a and b are the concentrations. By replacing the theoretical concentration of B in μg ml^{-1}, equally distributed around the mean value of 61·30 μg ml^{-1}, by their code values b, the following scheme is obtained:

Standard solution	Theoretical concentrations	
(B) in ml	B in μg ml^{-1}	b in code
2	35·03	−3
3	52·55	−1
4	70·06	+1
5	87·58	+3

The b values are chosen so that their sum (Σ b) is equal to zero.

To satisfy the equation

$$\text{Cov.} = \frac{\Sigma ab - (\Sigma a \times \Sigma b)/n}{n - 1} = 0,$$

the sum of the products of the concentrations of the two species (Σ ab) must also be zero. This condition obviously gives an infinite possibility to the choice of the a values. By adding the condition Σ a = 0, the choice of the a values becomes limited.

An arbitrary value of A in μg ml^{-1} is chosen in the scale of the measured solution of the tablets, the three other values are derived according to the code scheme −1, +3, −3, +1, so distributed that Σab = 0.

In the example the scheme is completed in the following way:

Theoretical concentrations		Standard solution		
a in code	A in μg ml^{-1}	(A) in ml	b	ab
−1	80·75	3·30	−3	+3
+3	122·35	5	−1	−3
−3	61·17	2·50	+1	−3
+1	101·55	4·15	+3	+3
Σa = 0			Σb = 0	Σab = 0

APPENDIX II: Calculation of the standard curves, taking into account interdependence of the two species.

The procedure described in the third analytical application is taken as an example.

Two measurements (L_I and L_{II}) are performed in four solutions, containing four concentrations of a and of b.

We assume that the current intensities (L_I and L_{II}), measured in each solution at the two reduction potentials, satisfy the following general equations:

$$L_I = Ax + By + C \quad (1)$$

$$L_{II} = A'x + B'y + C' \quad (2)$$

where x and y are the concentrations of each of the species and A B C and A′ B′ C′ are the parameters that must be determined (not to be confused with the symbols A and B used as concentrations).

The calculations to perform successively are:

1. Resolution of 2 sets of 3 equations with 3 unknown parameters:

$$\text{I.}\quad (\Sigma a^2)\ A + (\Sigma ab)\ B + (\Sigma a)\ C = \Sigma a.L_I \quad (3)$$

$$(\Sigma ab)\ A + (\Sigma b^2)\ B + (\Sigma b)\ C = \Sigma b.L_I \quad (4)$$

$$(\Sigma a)\ A + (\Sigma b)\ B + 4 = \Sigma L_I \quad (5)$$

$$\text{II.}\quad (\Sigma a^2)\ A' + (\Sigma ab)\ B' + (\Sigma a)\ C' = \Sigma a.L_{II} \quad (6)$$

$$(\Sigma ab)\ A' + (\Sigma b^2)\ B' + (\Sigma b)\ C' = \Sigma b.L_{II} \quad (7)$$

$$(\Sigma a)\ A' + (\Sigma b)\ B' + 4 = \Sigma L_{II} \quad (8)$$

where $\Sigma a = 0, \Sigma b = 0$ and $\Sigma ab = 0$.
$\Sigma a^2 = 20, \Sigma b^2 = 20$
$\Sigma L_I = 0{\cdot}83 + 1{\cdot}325 + 0{\cdot}787 + 1{\cdot}30 = 4{\cdot}242$
$\Sigma L_{II} = 1{\cdot}25 + 2{\cdot}08 + 2{\cdot}45 + 3{\cdot}33 = 9{\cdot}11$
$\Sigma aL_I = 2{\cdot}084$ and $\Sigma bL_I = 0{\cdot}872$
$\Sigma aL_{II} = 0{\cdot}97$ and $\Sigma bL_{II} = 6{\cdot}61$

In matrix symbolism we have:

$$M = \begin{matrix} -1 & -3 & 1 \\ +3 & -1 & 1 \\ -3 & +1 & 1 \\ +1 & +3 & 1 \end{matrix} \qquad M' = \begin{matrix} -1 & +3 & -3 & +1 \\ -3 & -1 & +1 & +3 \\ 1 & 1 & 1 & 1 \end{matrix}$$

and $$MM' = \begin{matrix} 20 & 0 & 0 \\ 0 & 20 & 0 \\ 0 & 0 & 4 \end{matrix}$$

the same matrix representing both sets of equations.

2. Transformation of the six equations into two equations with two unknown parameters, x and y, as represented by equations (1) and (2)

where $A = \frac{\Sigma a.L_I}{20} = 0{\cdot}1042$ $\quad A' = \frac{\Sigma a.L_{II}}{20} = 0{\cdot}0485$

$$B = \frac{\Sigma b.L_I}{20} = 0{\cdot}04362 \quad B' = \frac{\Sigma b.L_{II}}{20} = 0{\cdot}3305$$

$$C = \frac{\Sigma L_I}{4} = 1{\cdot}0606 \quad C' = \frac{\Sigma L_{II}}{4} = 2{\cdot}2775$$

which gives:

$$0{\cdot}1042\,x + 0{\cdot}04362\,y = L_I - 1{\cdot}0606 \quad (1)'$$

$$0{\cdot}0485\,x + 0{\cdot}3305\,y = L_{II} - 2{\cdot}2775 \quad (2)'$$

and after resolution:

$$x = 10{\cdot}2377\,(L_I - 1{\cdot}0606) - 1{\cdot}3513\,(L_{II} - 2{\cdot}2775) \quad (9)$$

$$y = -1{\cdot}5024\,(L_I - 1{\cdot}0606) + 3{\cdot}224\,(L_{II} - 2{\cdot}2775) \quad (10)$$

3. Calculation from equations (9) and (10), of concentrations x and y (in code values) for each of the four solutions.

4. Reconversion to the concentrations in μg ml^{-1} by resolution of the two following equations

$$[A] = (t.x) - u \quad (11)$$

$$[B] = (v.y) - w \quad (12)$$

where, in the example considered, the slopes t and v from the curves in code are:

$$t = \frac{122{\cdot}35 - 61{\cdot}17}{(+3) - (-3)} = 10{\cdot}1958;$$

$$v = \frac{87{\cdot}58 - 35{\cdot}03}{(+3) - (-3)} = 8{\cdot}7583$$

and the intersepts u and w are:

$$u = 10{\cdot}1958\,(+3) - 122{\cdot}35 = -91{\cdot}7625;$$

$$w = 8{\cdot}7583\,(\times 3) - 87{\cdot}58 = -61{\cdot}305$$

The results obtained are given in the following Table:

Hydrochlorothiazide

	Concentrations in μg ml^{-1}		
x	Recovered [A]	Theoretical A	%
−0·972	81·85	80·75	101·36
2·9737	122·08	122·35	99·78
−3·0341	60·82	61·17	99·44
1·0287	102·25	101·55	100·74

Dihydralazine

	Concentrations		
y	Recovered [B]	Theoretical B	%
−2·9662	35·32	35·03	100·84
−1·034	52·25	52·55	99·42
0·967	69·77	70·06	99·59
3·033	87·87	87·58	100·34