Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 6, pp. 625–633, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00
Pergamon Journals Ltd.

Short Communication

Development of reversed-phase HPLC assays for cyclodisone and clomesone

K. UMPRAYN, P. E. KENNEDY, J. C. LEE, W. WAUGH, C. M. RILEY and V. J. STELLA*

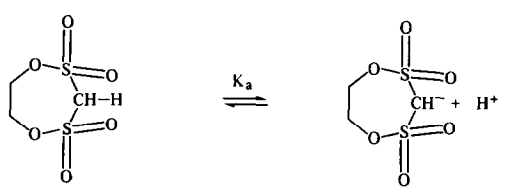
Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045, USA

Keywords: *Clomesone; cyclodisone; high-performance liquid chromatography; PRP-1 column; mobile phase effects; pK_a determinations; UV spectrophotometry; solubility; stability.*

Introduction

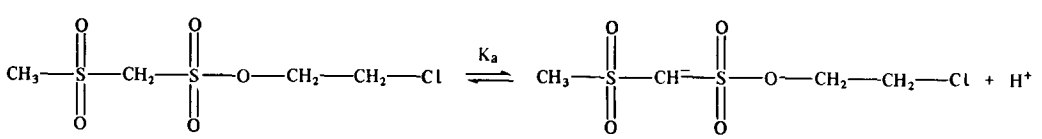
Cyclodisone (NSC 348948, 1,5,2,4-dioxadithiepane-2,2,4,4-tetraoxide, Scheme 1) and clomesone (NSC 338947, 2-chloromethylsulphonylmethanesulphonate, Scheme 2) are structurally related investigational antineoplastic agents with activity against a variety of tumours *in vitro* and in animal models [1]. Both drugs are to enter Phase 1 clinical trials in the near future.

Neither drug demonstrates significant UV absorption at wavelengths greater than 200 nm making their analysis by high-performance liquid chromatography (HPLC) difficult. However, both compounds have weakly acidic (pK_as > 9) methylene protons



Cyclodisone

Scheme 1



Clomesone

Scheme 2

*Author to whom correspondence should be addressed.

which are dissociated at high pH values (Schemes 1 and 2). The conjugate bases of both clomesone and cyclodisone exhibit end absorption which may be utilized in their determination by HPLC. Due to the high pH values necessary to ionize the compounds of interest, a macroporous copolymer of poly-(styrene–divinylbenzene) (PRP-1) was used rather than a reversed-phase column based on silica gel which is soluble in mobile phases having a pH of greater than 7.5. The pK_as of the two compounds were determined spectrophotometrically and these data were used in the interpretation of the chromatographic results.

Experimental

Chemicals and reagents

Cyclodisone and clomesone were provided by The National Cancer Institute, Bethesda, MD, USA and were used as received. All the aqueous buffers were prepared from reagent grade salts which were obtained from various sources. HPLC grade acetonitrile was obtained from Fisher Scientific, Fair Lawn, NJ, USA and the water was purified using the Mega-Pure system (Model MP-1, Corning, Buffalo, NY, USA).

Liquid chromatography

A modular liquid chromatograph was constructed from the following components: Altex 110A pump (Beckman, Berkeley, CA, USA), a Rheodyne model 7125 injector fitted with a 20 μl loop (Rheodyne, Cotate, CA, USA), a Spectroflow 783 detector (Kratos Analytical, Ramsey, NJ, USA), set at 214 nm, and a model B5117-1 Omniscribe recorder (Houston Instruments, Austin, TX, USA). The column (15 cm × 4.6 mm) contained 10 μm spherical macroporous poly-(styrene–divinylbenzene) copolymer (PRP-1, Hamilton Company, NV, USA). The column was packed at 6000 psi for 30 min using a slurry of PRP-1 (2.3 g) in 60 ml of chloroform and methanol. The initial slurry was degassed in an ultrasonic bath for 3 min immediately prior to packing.

The effect of mobile phase pH on the retention and detector response for clomesome and cyclodisone was studied. For cyclodisone, mobile phases containing 20% (by volume) acetonitrile in phosphate (pH 8.5), borate (pH values 9.5 and 10.0) and phosphate (pH values 11.1 and 11.5) buffers (all 0.06 M) were studied. For clomesone, mobile phases containing 22% (by volume) acetonitrile in carbonate (pH values 9.0 and 10.0) and phosphate (pH values 11.0 and 12) buffers (all 0.1 M) were studied. The solutes were injected as solutions (200 μg/ml) in the appropriate mobile phase. The void volume of the column (V_o) was determined for each mobile phase, by injection of a dilute $NaNO_2$ [2] in water. The capacity factors (k') were determined, at least in duplicate from the equation:

$$k' = (V_r - V_o)/V_o, \tag{1}$$

where V_r is the retention volume of the solute and V_o is the retention volume of $NaNO_2$. A flow rate of 1.0 ml/min was used throughout. Calibration curves were prepared ($n > 5$) by diluting stock solutions (10 mg/ml) of cyclodisone and clomesone in acetonitrile with mobile phase. Test solutions were diluted with mobile phase (if necessary) so that they fell within the linear ranges of the calibration curves. Both test and calibration solutions were injected in duplicate. The accuracy and precision of the procedures for each drug were determined by preparing solutions of known concen-

tration (clomesone: 0.2, 0.5 and 1 mg/ml; cyclodisone 50 and 100 μg/ml) and assaying each solution, three times in the case of clomesone and five times in the case of cyclodisone.

Spectrophotometry

The acid dissociation constants (K_a) of cyclodisone and clomesone were determined spectrophotometrically using a Cary 118 spectrophotometer (Varian Instruments, Palo Alto, CA, USA). The absorption (A) of cyclodisone (1.67×10^{-3}M) was determined at 217 nm in various buffers over the pH range of 7.01–12.01. Phosphate buffers (0.1 M) were used at pH values 7, 11 and 12 and borate buffers (0.1 M) were used over the pH range of 8.5–10.5. The ionic strength was adjusted to 0.5 with $NaClO_4$. The acid dissociation constant of clomesone (1.13×10^{-2} M) was determined in a similar manner at 225 nm. Due to the instability of the drugs, aliquots (100 μl) of stock solutions were mixed with the appropriate buffers in the 1 cm quartz cell and the absorbances measured immediately.

Stability studies

The stability of clomesone and cyclodisone was studied at 25 ± 0.1°C in various buffers (μ = 0.5) over the pH ranges of 2.7–11.6 for cyclodisone and 4.0–9.0 for clomesone. The temperature was maintained at 25 ± 0.1°C using a model 2095 Bath and Circulation water bath (Forma Scientific, Ohio, USA). Various buffers were used to maintain the pH and the ionic strength was adjusted to 0.5 with either NaCl (cyclodisone) or $NaClO_4$ (clomesone). The initial concentrations of both drugs were *ca* 10^{-3} M and the decomposition was followed for 3–4 half lives by periodic analysis of the solutions by HPLC. The pHs of the solutions were monitored periodically and found to change by less than 0.1 in all cases.

Solubility studies

The solubility of clomesone was studied by sonicating an excess of the drug in water for 60 min. The suspension was then filtered (0.45 μm), allowed to equilibrate to room temperature (23 ± 1°C) for 1 h and assayed by HPLC for clomesone. The solubility of cyclodisone was determined at various pH values in a similar manner. Phosphate buffers were used at pH values 8 and 11.5, carbonate at pH values 9.2 and 11.8 and acetate at pH 5. In each case the total buffer concentration was 0.04 M and the ionic strength was adjusted to 0.15 with NaCl.

Results and Discussion

pK_a *determinations*

The pK_as of cyclodisone and clomesone were determined from the absorption data at 217 nm and 225 nm, respectively. Two methods were used to evaluate the data. In the first method, the absorption data was fitted to equation (2) by non-linear least squares regression using the computer programme, MULTI:

$$A = (A_o[H^+] + A_\infty K_a)/([H^+] + K_a), \quad (2)$$

where $[H^+]$ is the hydrogen ion concentration, K_a is the dissociation constant and A_o and A_∞ are the absorbances of the unionized and ionized forms of the drugs, respectively. In

the second method, the data was fitted by least squares linear regression to equation (3) which is a linearized form of equation (2):

$$(A - A_0)[H^+] = K_a[A_\infty - A]. \quad (3)$$

The results of these analyses are shown in Table 1 and it can be seen that the agreement between the two methods is excellent. The average pK_a, obtained from the mean K_as by the two methods, was 10.74 for clomesone and 9.62 for cyclodisone.

Table 1
Dissociation constants for clomesone and cyclodisone obtained by spectrophotometry at 217 nm

	Cyclodisone K_a* ($\times 10^{10}$)	pK_a*	Clomesone K_a* ($\times 10^{11}$)	pK_a*
Method 1†	2.40	9.62	1.95	10.71
Method 2‡	2.29	9.64	1.66	10.78

*$pK_2 = -\log K_a$ (mean of two determinations).
†Equation (2).
‡Equation (3).

HPLC assay development

Cyclodisone and clomesone are essentially unionized and have negligible absorbance above 200 nm at pHs of less than 7.00 (Fig. 1) and basic mobile phases were needed to take advantage of the absorptivities of the respective conjugate bases. Consequently, a PRP-1 column was used rather than one based on silica gel which is soluble in water above pH 7.5. Various workers [2–4] have shown that PRP-1 columns are stable over a wide range of pH values (1–13) and Pietrzyk *et al.* [5] have shown that the mechanism of retention on ionic compounds may be explained in terms of the Stern–Gouy–Chapman theory of electrical double layers.

The effects of mobile phase pH on the detector response (Figs 2 and 3) and retention (Fig. 4) of cyclodisone and clomesone were investigated. A mobile phase composed of 0.06 M buffer: acetonitrile (80:20, v/v) was used for the chromatography of cyclodisone and one composed of 0.1 M buffer: acetonitrile (78:22, v/v) in the case of clomesone. The capacity ratios were determined using equation (1). The void volume (V_0) was independent of mobile phase composition and the value of 1.42 ± 0.02 ml was in good agreement with reports by Lee and Kindsrater [6]. The retention of both clomesone and cyclodisone decreased with increasing pH; however at any particular pH, over the range 8–11, clomesone was more strongly retained than cyclodisone (Fig. 4). Neither compound was retained to any extent ($k' < 0.2$) above pH 12. On the other hand, sensitivity increased with increasing pH due to the increase in the fraction of chromphoric species present (Figs 2 and 3). However, it should be noted that poor peak shape for cyclodisone was observed above a pH of 11.1, resulting in increased peak areas but decreased peak heights with increased pH above this value (Fig. 2). The effects of pH on both retention and sensitivity may be related to the differences in the pK_as of cyclodisone and clomesone. Thus, mobile phases of different pH values were required for the analysis of the two drugs, each providing the necessary compromise between

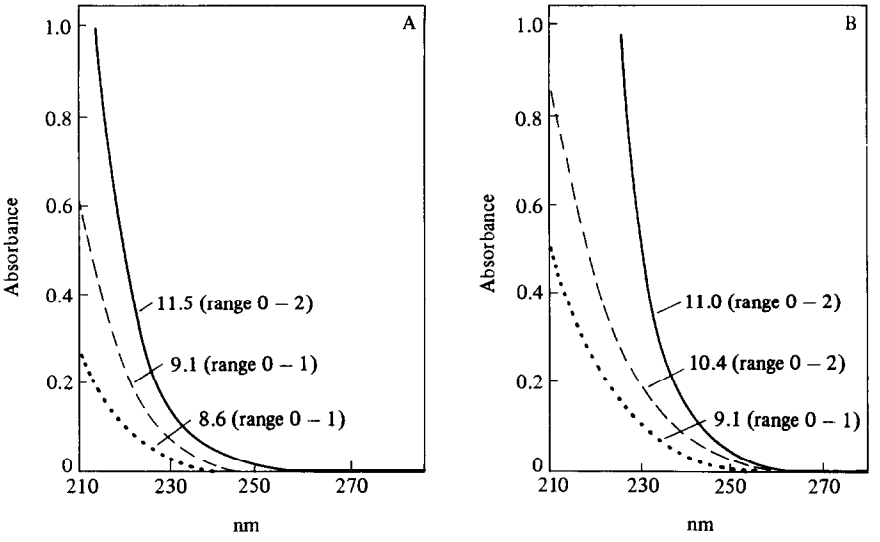


Figure 1
UV spectra of cyclodisone (A) and clomesone (B) at various pHs.

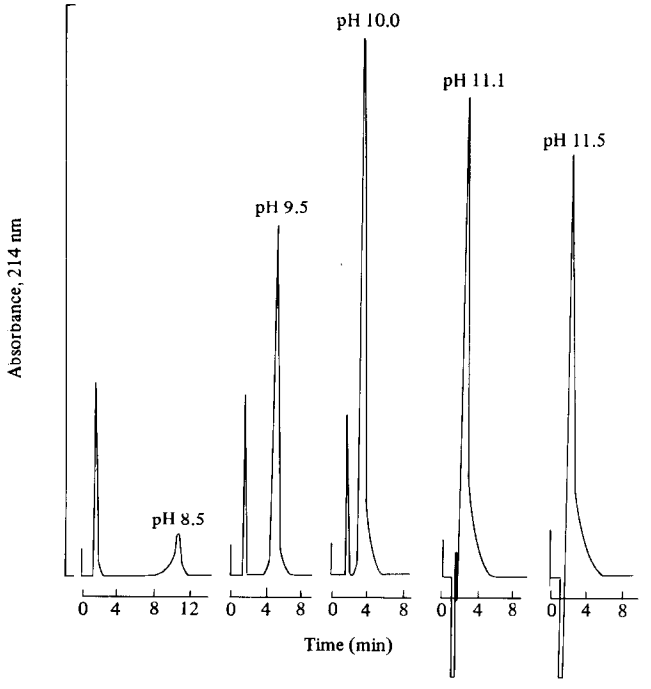


Figure 2
Chromatograms of cyclodisone at various pH values [key: pH 8.5 (phosphate), 9.5 and 10 (borate), pH 11.1 and 11.5 (phosphate), mobile phase was 0.06 M aqueous buffer solution: acetonitrile (80:20)].

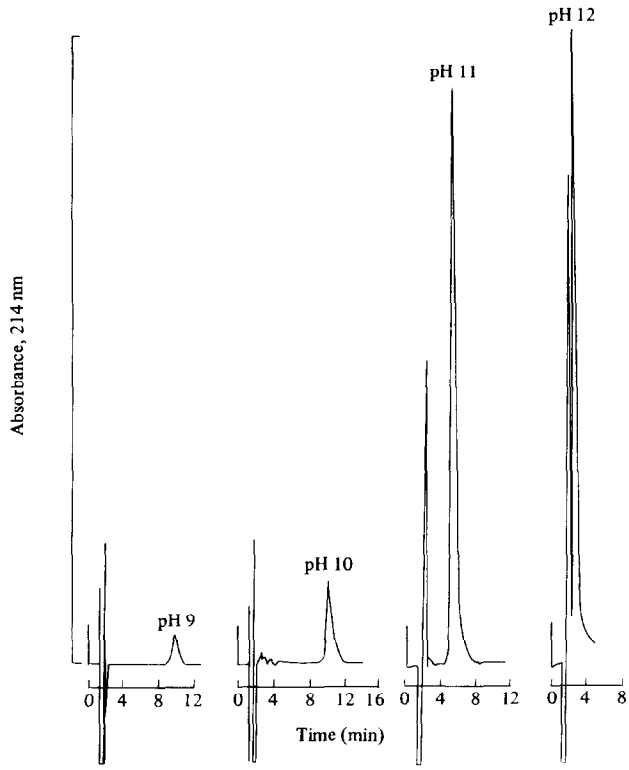


Figure 3
Chromatograms of clomesone at various pH values [key: pH 9 and 10 (carbonate), pH 11 and 12 (phosphate), mobile phase was 0.1 M aqueous buffer solution: acetonitrile (78:22)].

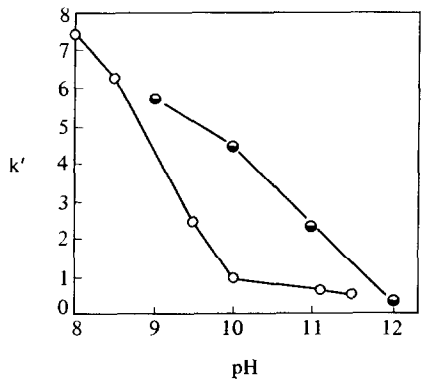


Figure 4
Influence of the mobile phase buffer pH on the capacity factor (k') of cyclodisone (○) and clomesone (◒).

adequate retention and detector sensitivity. Phosphate buffer (0.1 M, pH 11): acetonitrile (78:22) was preferred for the analysis of clomesome and borate buffer (0.06 M, pH 9.5): acetonitrile (80:20) was preferred for the analysis of cyclodisone. These conditions gave virtually identical retention volumes (5 ml) for the two compounds and the sensitivity needed for the stability and solubility studies.

The relationships between peak heights and concentration injected were linear ($r > 0.99$) for clomesone over the range 0–1.0 mg/ml and cyclodisone over the range 0–0.2 mg/ml. The precisions and accuracies for the assays for both drugs were satisfactory and the results of these studies are summarized in Table 2.

Table 2
Precision and accuracy of the HPLC assays for the determination of clomesone and cyclodisone

Drug	Concentration (mg/ml)	Precision* (%)	Accuracy† (%)
Clomesone			
	0.2	0.50	96.5
	0.5	1.15	98.6
	1.0	0.56	100.6
Cyclodisone			
	0.05	3.07	97.9
	0.10	2.10	97.9

*Relative standard deviation expressed as a percentage of the mean ($N = 3$).
†Concentration found expressed as a percentage of the known concentration.

Applications

The applicability of the analytical methodology to pre-formulation studies was demonstrated in the following solubility and stability studies. The intrinsic solubility of clomesone in water was found to be 7.5 mg/ml. Since this was greater than the concentration required for the final intravenous injection, further studies on clomesone were not conducted. The solubility of cyclodisone in aqueous solutions was studied as a function of pH (Table 3) and it can be seen that the intrinsic solubility (pH 5–9.2) is around 1 mg/ml and that the apparent solubility increases at higher pH values due to ionization of the methylene group (Scheme 1). However, pH values approaching 11 are needed to achieve a solubility of greater than 5 mg/ml which is the desired formulation goal.

The degradation of cyclodisone (Fig. 5) and clomesone (Fig. 6) was pseudo-first order over at least 3 half-lives. No degradation products were observed indicating that these compounds do not possess the ionizable methylene groups present in clomesone and cyclodisone. An alternative explanation is that the degradation products did not elute from the column. In either case, it is clear that the present methods are stability indicating. The data presented here are to demonstrate the applicability of the analytical methods to stability studies. Further work is in progress to elucidate the mechanism of degradation of clomesone and cyclodisone and to develop the complete pH–rate profiles after correction for buffer effects. The results of these studies will be presented elsewhere.

Table 3
Apparent solubility of cyclodisone in various buffers

Buffer*	pH	Solubility (mg/ml)†
Acetate	5.0	1.16
Phosphate	8.0	0.96
Carbonate	9.2	1.04
	10.0	1.58
	10.8	2.66
Phosphate	11.0	5.60

*Buffer concentration = 0.06 M (μ = 0.15 with NaCl), 23 ± 1°C.
†Apparent solubility after ultrasonification for 1 h.

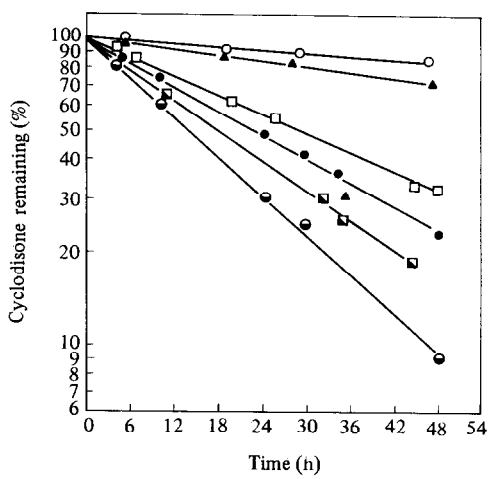


Figure 5
Degradation of cyclodisone at various pH values and 25°C (μ = 0.5 with NaCl and buffer concentration of 0.06 M) [key: ○, pH 11.6 (phosphate); ▲, pH 11.2 (phosphate); □, pH 10.5 (carbonate); ●, pH 2.66 (HCl); ◪, pH 5.0 (acetate); ◒, pH 7.45 (phosphate)].

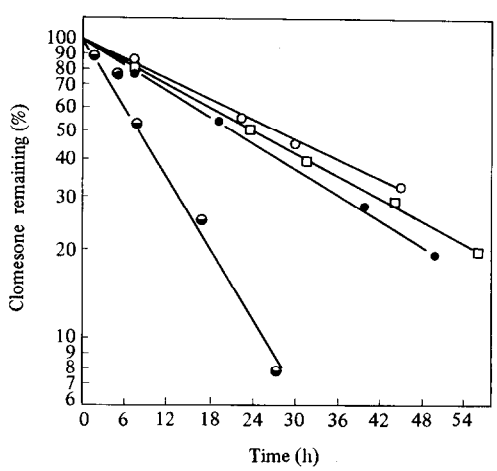


Figure 6
Degradation of clomesone at various pH values and 25°C (μ = 0.5 with $NaClO_4$ and buffer concentration of 0.06 M) [key: ○, pH 4.0 (acetate); □, pH 5.0 (acetate); ○, pH 7.7 (phosphate); ◒, pH 9.0 (carbonate)].

Acknowledgements: This work was supported by the National Cancer Institute Contract NO1-CM-37502 and NO1-CM-67912.

References

[1] N. R. Lomax and V. L. Narayanan, in *Chemical Structures of Interest to the Division of Cancer Treatment*, Vol. IV, pp. 22–23. National Cancer Institute, Bethesda, MD (1984).
[2] D. P. Lee, *J. Chromatogr.* **20**, 203–208 (1982).
[3] E. P. Krocff and D. J. Pietrzyk, *Anal. Chem.* **50**, 505–512 (1978).
[4] R. G. Baum, R. Saetre and F. F. Cantwell, *Anal. Chem.* **52**, 15–19 (1980).
[5] D. J. Pietrzyk, E. P. Kroeff and T. D. Rotch, *Anal. Chem.* **50**, 497–502 (1978).
[6] D. P. Lee and J. H. Kindsrater, *Anal. Chem.* **52**, 2425–2428 (1980).

[*First received for review 30 January 1987; revised manuscript received 6 April 1987*]