



ELSEVIER

Journal of Chromatography B, 696 (1997) 303–306

JOURNAL OF  
CHROMATOGRAPHY B

Short communication

## Determination of diazolidinyl urea in a topical cream by high-performance liquid chromatography

Robert O. Williams, III\*, Vorapann Mahaguna, Mongkol Sriwongjanya

Pharmaceutics Division, College of Pharmacy, University of Texas, Austin, TX 78712-1074, USA

Received 7 January 1997; revised 25 March 1997; accepted 8 April 1997

### Abstract

A high-performance liquid chromatographic method for the determination of diazolidinyl urea (DU) in a cream formulation is described. The aqueous phase of the emulsion was separated by centrifugation, removed, filtered, diluted and applied onto the HPLC system. DU was detected by ultraviolet absorption at a wavelength of 214 nm. The calibration curve was linear over the range of 79–553  $\mu\text{g/ml}$ , and identical when determined on consecutive days. The relative standard deviation for repeat determinations was less than 0.5%. Recoveries were 97.74–101.72%. This analytical method is useful for quantitation of DU in cream formulations. © 1997 Elsevier Science B.V.

**Keywords:** Diazolidinyl urea

### 1. Introduction

Diazolidinyl urea (DU) is used as an antimicrobial preservative in many topical pharmaceutical and cosmetic formulations. DU is an antibacterial agent active against Gram-positive and Gram-negative species, including *Pseudomonas* species [1–3]. Also, it reportedly has some activity against yeast and mold [4]. DU often is used synergistically in combination with methylparaben and propylparaben where the combination provides protection against bacterial, yeast and mold contamination during product use [5,6]. The structure of DU is shown in Fig. 1. DU is a white, fine, free-flowing powder. The solubility of DU is 230 g in 100 g of water and less than 0.01 g in 100 g of mineral oil, therefore, it is primarily

solubilized in the aqueous phase of the emulsion preparation.

A quick, easy and reliable method for the determination of DU is desirable. High-performance liquid chromatography (HPLC) with UV detection is an advantageous technique because of its separation capability and suitability for routine analytical analysis. DU is recommended [1] to be used in the range of 0.1 to 0.3% in topical preparations, therefore, its determination and quantification is not trivial

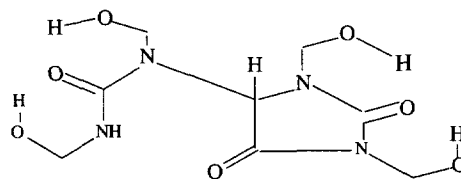


Fig. 1. Structure of diazolidinyl urea.

\*Corresponding author.

at these low concentrations. A review of the literature indicated that no specific method has been developed and reported for the determination of DU in pharmaceutical or cosmetic topical formulations. Also, the topical preparations containing DU are in combination with a complex mixture of other components that are present in much higher concentrations and may interfere with the detection of DU.

The objective of this study was to develop a HPLC method appropriate for measuring DU in an experimental topical cream formulation. This technology should be applicable to other similar complex formulations.

## 2. Experimental

### 2.1. Chemicals

The following materials were used as received for the preparation of the experimental cream formulation: mineral oil (Spectrum, Gardena, CA, USA); sorbitan monostearate (ICI Americas, Wilmington, DE, USA); stearyl alcohol (CPC International, Edison, NJ, USA); cetyl alcohol (Spectrum); sodium lauryl sulfate (Fluka, Ronkonkoma, NY, USA); diazolidinyl urea (Sigma, St. Louis, MO, USA); methylparaben (Spectrum); propylparaben (Spectrum) and triethanolamine (EM Science, Gibbstown, NJ, USA).

Methanol (EM Science) was HPLC grade and used as received. Purified water was used (Milli QUV Plus water system; Millipore, Molsheim, France).

### 2.2. Chromatographic conditions

The chromatographic system (Shimadzu, Columbia, MD, USA) consisted of a solvent delivery module (Model LC 9A), variable-wavelength UV spectrophotometric detector (Model SPD-6A), chromatographic data control and acquisition system (CLASS-VP), and LC–personal computer (PC) interface (Model LPI 6B). A Supelcosil LC-CN (Supelco, Bellefonte, PA, USA) cyanopropyl bonded phase column (15 cm × 4.6 mm, 5 μm) which has silica as the stationary phase and cyanopropylsilyl as the bonded phase was used.

The mobile phase was purified water–methanol

(80:20, v/v) which was degassed and filtered prior to use. The flow-rate was 1 ml/min and the injection volume was 20 ml. The absorbance was monitored at 214 nm. The run time was 15 min. Replicate standards were injected to ensure repeatability prior to sample analysis. System suitability criteria were established: relative standard deviation (R.S.D.) of ten replicate injections, ≤2.0%; number of theoretical plates, >900 plates/column; and tailing factor for the DU peak, ≤2. A check standard of known concentration (0.277 mg/ml) was inserted between every five samples.

### 2.3. Sample preparation

The experimental cream formulation contained either 0.25% or 0.34% (w/w) DU, and was prepared as follows. The aqueous phase was prepared by admixing and heating to 75°C the sodium lauryl sulfate (0.60%, w/w), methylparaben (0.20%, w/w), propylparaben (0.02%, w/w), triethanolamine (3.00%, w/w), DU (formulation A: 0.25%, w/w; formulation B: 0.34%, w/w) and purified water (formulation A: 68.53, w/w; formulation B: 68.45%, w/w). The oil phase was prepared by combining and heating to 70°C the mineral oil (20%, w/w), sorbitan monostearate (0.40%, w/w), stearyl alcohol (6.00%, w/w) and cetyl alcohol (1.00%, w/w). The oil phase was added to the aqueous phase with continuous agitation to form the oil-in-water emulsion. The emulsion was homogenized by passing through a colloid mill. DU was deleted from the cream formulation for validation purposes.

Aliquots of the experimental cream formulation were weighed and placed into a vortex tube and centrifuged at 1500 g for 4 h to separate the aqueous and oil phases. The aqueous phase was removed from the sample tube, filtered through a 0.22 μm cellulosic filter (Gelman Sciences, Ann Arbor, MI, USA), and serially diluted with purified water for HPLC analysis. The samples were diluted such that the final concentration was within the linear portion of the standard curve for DU.

## 3. Results and discussion

A HPLC method with UV detection was developed for quantification of DU in an experimental

cream formulation. A wavelength of 214 nm was chosen because DU had good molar absorptivity at this level allowing for sample dilutions to be made. DU had a maximum absorbance wavelength at 236 nm. No interference was found at the wavelength of 214 nm between the components of the cream formulation and DU.

The HPLC chromatogram shown in Fig. 2 shows the excellent separation of DU from the other components of the complex emulsion formulation. The sample diluent and sample placebo showed no interferences in the region of interest, and excellent method specificity was demonstrated. The results shown in Fig. 2 also illustrate the components that eluted from the column and their retention times. The order of elution and retention times were sodium lauryl sulfate (1.20 min), triethanolamine (1.25 min), diazolidinyl urea (2.02 min), methylparaben (4.89 min) and propylparaben (9.91 min).

Validation data were generated for this analytical method with the experimental cream formulation. The results presented in Table 1 indicated that the linearity of the calibration curve was excellent. Three five point standard curves were generated on three different days. The curves obtained were linear as indicated by the  $r^2$  of 0.9997, 0.9998 and 0.9998 for Days 1, 2 and 3, respectively. A fit of a least squares line to the experimental results yielded an intercept of  $-47\,729$ ,  $-47\,819$  and  $-39\,395$  for Days 1, 2 and 3, respectively. The results shown in Table 2 describe the repeatability of the determinations. The

Table 1  
Diazolidinyl urea standard curves determined on three consecutive days

Concentration (mg/ml)	Area counts standard curve		
	Day 1	Day 2	Day 3
0.079	442 737	441 388	443 240
0.138	796 070	781 709	797 264
0.277	1 599 279	1 595 443	1 595 842
0.415	2 457 224	2 443 045	2 454 056
0.553	3 316 682	3 289 175	3 289 590
$r^2$	0.9997	0.9998	0.9998
y-intercept	$-47\,729$	$-47\,819$	$-39\,395$
$S_{xx}^2$	21 947	16 928	18 181

<sup>a</sup> Standard error of the estimate.

concentration of the standard solution of DU employed to verify the system suitability was 0.553 mg/ml. The precision of the chromatographic method was determined by making ten replicate injections of a working standard solution on three consecutive days. The precision was excellent as indicated by the low magnitudes of the R.S.D.s on Day 1, Day 2 and Day 3 of 0.49%, 0.48% and 0.26%, respectively. The instrument precision for the working standard solution was well below the 2% R.S.D. limit for DU.

Studies of DU recovery were performed to determine the accuracy of the method. The recovery of DU from spiked samples of the experimental cream formulation is presented in Table 3. The data indicated excellent recovery for the two levels of DU

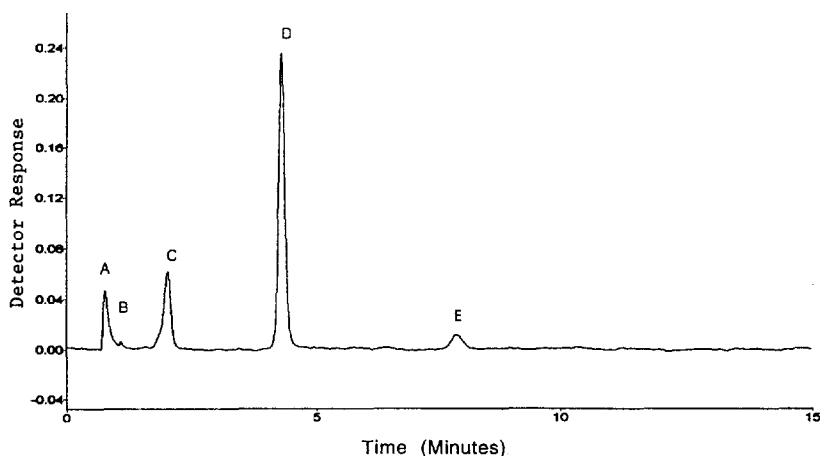


Fig. 2. Typical HPLC chromatogram of the experimental cream formulation containing diazolidinyl urea (A=sodium lauryl sulfate; B=triethanolamine; C=diazolidinyl urea; D=methylparaben and E=propylparaben).

Table 2  
Repeatability of the diazolidinyl urea measurements in the cream preparation

Injection No.	Area (recovery, %)		
	Day 1	Day 2	Day 3
1	3 314 175 (100.39)	3 284 050 (100.18)	3 304 663 (100.69)
2	3 321 637 (100.61)	3 306 768 (100.86)	3 298 744 (100.51)
3	3 310 483 (100.28)	3 263 422 (99.56)	3 292 376 (100.32)
4	3 315 936 (100.44)	3 296 377 (100.55)	3 279 715 (99.94)
5	3 297 905 (99.90)	3 298 979 (100.63)	3 280 881 (99.97)
6	3 289 844 (99.66)	3 312 527 (101.03)	3 292 780 (100.33)
7	3 267 466 (98.99)	3 309 011 (100.93)	3 298 588 (100.51)
8	3 299 065 (99.93)	3 280 726 (100.08)	3 286 006 (100.13)
9	3 305 165 (100.12)	3 286 856 (100.26)	3 281 730 (100.00)
10	3 287 898 (99.60)	3 308 534 (100.91)	3 285 620 (100.12)
Average	3 300 957 (99.99)	3 294 725 (100.50)	3 290 110 (100.25)
S.D.	16 206 (0.48)	15 738 (0.47)	8 634 (0.26)
R.S.D. (%)	0.49 (0.48)	0.48 (0.47)	0.26 (0.26)

Table 3  
Recovery from spiked placebo samples

Theoretical concentration (mg DU/g cream)	Active concentration detected (mg DU/g cream)	Recovery (%)
2.50	2.49	99.88
2.50	2.54	101.72
3.40	3.32	97.74
3.40	3.47	101.94

investigated. The recovery was 99.88% and 101.72% of the amount of DU theoretically added to the sample for the 0.25% (w/w) concentration, and 97.74% and 101.94% of the amount theoretically spiked in the sample for the 0.34% (w/w) concentration.

The simplicity and versatility of this HPLC method has been demonstrated in this investigation using an experimental water-in-oil emulsion cream formulation. The method was shown to be sensitive, specific, precise and accurate for the HPLC analysis of DU in the experimental cream formulation containing a complex mixture of components. The HPLC method may be useful for other pharmaceutical and cosmetic topical preparations containing DU.

## References

- [1] P.A. Berke, D.C. Steinberg, W.E. Rosen, *Cosmet. Toiletries* 97 (1982) 89–93.
- [2] P.A. Berke, W.E. Rosen, *Cosmet. Toiletries* 97 (1982) 49–53.
- [3] E.M. Jackson, *Cosmet. Derm.* 8 (1995) 27–30.
- [4] I. Cagliani, *Boll. Chim. Farm.* 124 (1985) 356–364.
- [5] P. Berke, W. Rosen, *J. Soc. Cosmet. Chem.* 31 (1980) 37–40.
- [6] C.B. Anger, D. Rupp, P. Lo and H. Takruri, in H.A. Lieberman, M.M. Rieger and G.S. Banker (Editors), *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Marcel Dekker, New York, 2nd ed., 1996, pp. 377–435.