Original Article

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Initial characterization of D-cycloserine for future formulation development for anxiety disorders

Gagan Kaushal[1,*], Ronaldo Ramirez[1], Demelash Alambo[1], Wacharah Taupradist[1], Krunal Choksi[1], Cristian Sirbu[2]

[1] *School of Pharmacy, University of Charleston, Charleston, WV, USA;*
[2] *Department of Behavioral Medicine and Psychiatry, School of Medicine, West Virginia University, Charleston Division, Charleston, WV, USA.*

ABSTRACT: The purpose of this study is to characterize D-cycloserine (DCS) physico-chemical properties to facilitate future formulation development of DCS for anxiety disorders. A stability-indicating HPLC assay method for the quantitation of DCS was developed and calibrated to be used for this study. The partition coefficient was determined and compared with the predicted value. The solution stability of DCS was studied under various pH (2-11.5) and ionic strengths of 10 and 20 mM at physiological temperature of 37°C. The 250 mg capsule was compounded to the nominal strength of 50 mg used for anxiety disorders. These capsules were then put under stability. The *in vitro* dissolution was also carried out at 37°C as per the United States Pharmacopeia (USP) guidelines. The partition coefficient value (Kp) determined for the DCS was log Kp = –2.89 ± 0.06 (*n* = 6). The pH-solution stability profile shows that DCS has maximum stability under alkaline conditions. The maximum rate of degradation was seen at pH of 4.7. The mean percent recovery of DCS from the capsules compounded to strength of 50 mg was 100.3 ± 1.4. The stability study of the reformulated capsules concluded that reformulated DCS is stable for at least one year at room temperature. The *in vitro* dissolution illustrates that all the DCS is released from the capsules in 10 min. The present characterization of DCS study will serve as guidance for the future directions regarding the reformulation of DCS in order to be used in anxiety disorders.

Keywords: D-Cycloserine, HPLC, stability, pH-solution stability, anxiety, formulation, physicochemical properties

1. Introduction

D-Cycloserine (DCS), an FDA approved drug under the name Seromycin® (D-cycloserine capsules, USP, 250 mg), (*R*)-4-amino-1,2-oxazolidin-3-one, is a broad-spectrum antibiotic that is produced by a strain of *Streptomyces orchidaceus* and has also been synthesized (Figure 1). It is used as a second line treatment for tuberculosis. Besides of that, *in vitro* studies have demonstrated that DCS has a high affinity, high efficacy partial agonist with moderate specificity for strychnine-insensitive excitatory (GLY-B) glycine site of the *N*-methyl-D-aspartate (NMDA) receptor (*1,2*). Based on its action on the NMDA receptors, recently this drug has been used on numerous clinical trials as an enhancer of exposure therapy for the treatment of anxiety disorders (*3-8*). DCS has been shown to facilitate exposure treatment and its consequential extinction learning and fear reduction in animal and human studies. Studies of acrophobia (fear of heights; (*4*)), social phobia/social anxiety disorder (*3*), and obsessive-compulsive disorder (*9,10*) have shown more rapid extinction learning and fear reduction with DCS when compared to placebo.

DCS first entered the market in 1952 and since the commercial introduction of DCS; very few studies have examined the physicochemical properties of this drug (*11,12*). Some of its physicochemical properties are listed in Table 1. In order to be used as an enhancer of exposure therapy for the treatment of anxiety disorders, the possibility of using this drug in different formulations, other than capsules, as well as using alternate routes of administration needs to be explored to maximize efficacy.

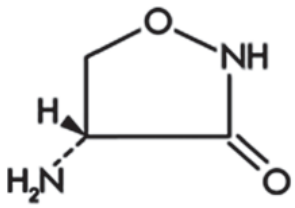

Figure 1. The structural formula of D-cycloserine.

**Address correspondence to:*
Dr. Gagan Kaushal, School of Pharmacy, University of Charleston, Charleston, WV 25304, USA.
e-mail: gagankaushal@ucwv.edu

Table 1. Physicochemical properties of D-cycloserine

Physicochemical parameter	DCS values (*16*)	ACD/PhysChem predicted values
Aqueous solubility	> 1 mg/mL	1,000 mg/mL
Partition coefficient	–1.631	–2.99
Molecular weight	102.09	102.09
Melting point	147°C	–
pH of saturated aqueous solution	5.5-6.5	–

Very few studies have explored these possibilities. The application of DCS through the nose mucosa for delivery to the brain was done by Musumeci *et al*. (*13*). This drug is very hydrophilic in character and cannot easily pass through the blood-brain barrier. Thus the nasal delivery gives the advantage of delivering this drug to the CNS and eliminates the need for systemic delivery. In their study (*13*), DCS liposomes were formulated for nasal delivery. The stability of the final formulation at different temperature (25, 37, and 60°C) and pH (5.0, 7.4, and 9.0) was studied by these researchers. In another recently published study by the same group (*14*), the feasibility of using DCS loaded w/o nanocapsules for intranasal delivery was explored as well.

DCS needs to be well characterized with the present technology before any new formulation can be developed for this compound. It is very important to know its stability under different conditions. These studies were done back in 1950s and 60s and investigated the stability of DCS at different pH values in aqueous solutions (*12,15,16*). The studies found that DCS is stable under alkaline conditions and is easily degraded under the acidic conditions. The conditions used for these studies and the analogues that resulted after degradation is inconsistent. No study so far has used a stability-indicating HPLC method to monitor the degradation peaks. The degradation peaks can be monitored easily by using an HPLC method and can help better understand the process of degradation. Knowing these conditions would be very important to further develop any formulation of DCS. Thus one of our objectives of this investigation is to study the effect of pH, ionic strength, and temperature on the stability of DCS using a stability indicating HPLC method.

An important challenge in studies examining DCS efficacy as an enhancer of exposure therapy for anxiety disorders is identifying the doses that are associated with the highest therapeutic efficacy. DCS is commercially available as a 250 mg capsule. However on examining the eight clinical trial studies that use DCS in patients with anxiety disorders, a dose of 50 mg is commonly used. In order to give these doses to the patients, the 250 mg capsule is reformulated to 50 mg capsules. It is very important to make sure that no degradation has taken place and the correct strength of DCS is being administered to the patient. Thus, in the present study, we have investigated the stability of 50 mg compounded DCS capsules and also analyzed the *in vitro* dissolution of these capsules.

2. Materials and Methods

2.1. *Materials*

D-Cycloserine was obtained from Research Products International Corp. (Mt. Prospect, IL, USA) and was used without further purification. All chemicals, buffer reagents, and solvents used were of analytical grade and were purchased from Fisher Chemicals (Fair Lawn, NJ, USA). HPLC grade water and acetonitrile were also purchased from Fisher Chemicals (Fair Lawn, NJ, USA) and used throughout this study.

Lactose and gelatin capsules size#3 used for the compounding in the present study were purchased from Professional Compounding Centers of America (PCCA, 9901 South Wilcrest Drive, Houston, TX, USA) and are approved for human consumption. The DCS capsules (Seromycin®, 250 mg) used for compounding was procured from our local pharmacy and was purchased by the pharmacy from The Chao Center (Purdue Research Park, 3070 Kent Avenue, West Lafayette, IN, USA).

2.2. *HPLC method for DCS analysis*

For the analysis of DCS in unknown samples, a stability-indicating HPLC method for the separation and the detection of DCS in aqueous media was developed. All the chromatographic studies were performed on a Dionex Ultimate 3000 HPLC system connected with an absorbance detector. The separations were performed on Atlantis T3 C18 cartridge column (250 × 4.6 mm I.D., Waters Associates, Milford, MA, USA) with the column particle diameter of 5 µm. Column effluents were monitored at the wavelength of 220 nm for a run time of 5.0 min at the temperature of 30°C. For the mobile phase, 90% of 10 mM sodium phosphate buffer (pH 7.5) and 10% acetonitrile was used. The mobile phase was filtered and degassed before use. The flow rate was 0.75 mL/min with the injection volume of 10 µL.

2.3. *DCS standard curve*

The calibration of HPLC system was performed by constructing a standard curve using seven known concentrations of DCS. In order to prepare the standard curve, fixed amounts (25, 50, 100, 250, 500, 750, and 1,000 µg/mL) of standard DCS were respectively added to the HPLC grade water and these samples were analysed by using the standardized HPLC conditions.

Three quality control samples (30, 150 and 700 µg/mL) were processed and each of these samples was analyzed three times (inter-day variation) on three different days (intra-days variation). These were labeled as LQC (30 µg/mL), MQC (50 µg/mL), and HQC (700 µg/mL). The accuracy was calculated at each concentration as the ratio of the measured concentration to the nominal concentration multiplied by 100%.

The limit of quantitation (LOQ) of the method was defined as the lowest concentration that could be quantitatively determined with an acceptable precision and accuracy. The acceptable limits were defined as accuracy of 80-120% and precision of ≤ 20%.

2.4. *Stability-indicating assay and validation*

The suitability of the present HPLC conditions to be used as a stability-indicating method was tested by forced degradation of DCS. The forced degradation of all the samples was performed at an initial drug concentration of 500 µg/mL and was done under acidic and alkaline conditions. Acid hydrolysis was performed in a solution of pH of 1.5 adjusted with 1 M HCl and alkaline condition was carried out at pH of 12.5 adjusted with 1 M NaOH. Each of these two extreme pH solutions was prepared in triplicate. All the solutions were heated at 90°C for at least 4 h and the samples were withdrawn every hour. In order to see the effect of light, a standard concentration was also prepared and was stored at room temperature under normal fluorescent light. Each sample was analysed by HPLC using the standard conditions as explained above.

2.5. *Determination of partition coefficient*

The partition coefficient of DCS was determined in octanol against water. Water saturated with octanol was prepared by equilibrating 5 mL each of octanol and water by gentle stirring for 24 h using wrist action shaker (Model 75, Burrell Scientific, Pittsburgh, PA, USA) at room temperature. This solvent mixture was allowed to settle for 2 h and then 5 mg of DCS was added to this mixture in a 50 mL tube. The tube was again shaken using wrist action shaker for 24 h and was then left at room temperature for at least 2 h. A sample of 500 µL was taken from both the organic and aqueous phase for HPLC assay. The partition coefficients were then calculated as the ratio of the tested compound concentration in organic to that in the aqueous phase. The same procedure was followed by replacing the water phase with phosphate buffer saline (PBS, pH 7.4).

2.6. *pH-solution stability*

The stability study was designed as per a previous study done by Claudius *et al.* (*17*) on vancomycin with some modifications. DCS solution stability was analyzed under various pH (2.0-11.5) and ionic strength (10-20 mM) conditions at physiological temperature of 37°C. The buffers used for this study were as follows: 0.01 N HCl (pH 2.0), acetate (pH 4.7), phosphate (pH 7.0), Tris-HCl (pH 8.5), and phosphate (pH 11.5). The ionic strength was held constant at each buffer concentration by adjusting with sodium chloride. In addition, the stability was also analyzed in simulated gastric (SGF) and intestinal (SIF) fluids.

DCS samples were prepared in triplicate at a concentration of 500 µg/mL by dissolving it in the desired buffer in 25-mL type I clear glass vials (Wheaton Glass, Wheaton, IL, USA). The vials were then sealed with Teflon-coated butadiene stoppers and further covered with Parafilm® to minimize evaporation. Each of the vials was further wrapped with aluminum foil in order to protect from the light. Samples were stored in a static oven at 37°C and 1-mL samples were withdrawn, filtered and analyzed at 0, 1, 7, 15, 22, and 30 days, respectively.

2.7. *Reformulation of 50 mg DCS capsules*

Seromycin® (D-cycloserine) is available as a 250 mg capsule and was compounded to the nominal strength of 50 mg per capsule using standard compounding techniques following the USP (Chapter <1075>) and GMP guidelines. The capsules were manually filled using ProFill 100 Capsule Filling Machine (Capsugel, 535 North Emerald Road, Greenwood, SC, USA), which can fill 100 capsules at one time. At least six capsules were withdrawn from a batch of 100 capsules and were tested for weight variation test as per the USP guidelines (Chapter <905>) on uniformity of dosage units. For the assay analysis, three capsules were randomly withdrawn from the batch of compounded capsules and were assayed for the active content by the stability indicating HPLC procedure as outlined above.

2.8. *In vitro dissolution of DCS capsules*

In vitro dissolution is one of the most important tools that should be carried out in order to predict the *in vivo* performance of any dosage form. The *in vitro* dissolution was carried out at 37°C using Distek Dissolution System 2100C (Distek, Inc., North Brunswick, NJ, USA) as per the USP 32 guidelines. The dissolution media used for the studies was phosphate buffer (pH 6.8) and was prepared as per the USP guidelines. The USP dissolution Apparatus I set at speed of 100 rpm with 900 mL of buffer was used for this study. Both, 50 and 250 mg capsules of DCS were used for the dissolution study.

Samples (10 mL) of dissolution medium were removed at regular time intervals for up to 30 min. An equal volume of dissolution medium at 37°C was added to maintain a constant volume. The samples were prepared and the drug concentration was quantified by the standardized HPLC method outlined above. Six capsules were used for this study to make the data statistically significant and this experiment was repeated on three different days.

2.9. *Data analysis*

Statistical analysis was performed using Student's *t*-test or Two Way ANOVA. $p < 0.05$ was indicative of a significant difference.

3. Results

3.1. *Standard curve and method validation*

The chromatogram of DCS standards shows a peak at retention time of 3.5 min (Figure 2). A blank sample was also injected to the HPLC system and no peak was observed from this sample. As shown in Figure 3, a good linearity was exhibited in the concentration range (25-750 µg/mL) by using the presently developed HPLC method. The average coefficient of determination of 0.99 was observed for the standard curve. The slopes of the curves illustrated an excellent agreement with coefficient of variability.

The % R.S.D. values for intra-day precision study were < 1.0% and for inter-day study were < 2.0%, confirming that the method was sufficiently precise. An acceptable precision and accuracy was acquired by this method for all the standards and quality controls based on the recommended criteria (*18*). The percentage recovery of DCS using the present HPLC method was also calculated from the peak areas obtained. As shown from the data in Table 2, an admirable recovery was obtained at each of the added concentration. In accordance to the official requirements the limit of quantitation (LOQ) for the present method was 30 µg/mL.

3.2. *Stability-indicating HPLC method characterization*

One of our major goals was to develop a stability-indicating method for the detection of DCS. As soon as the DCS was added to a low pH solution, a significant degradation was observed. As per Figure 4A, a degradation peak was seen at the retention time of 3.39 min and there was a significant decrease in the DCS peak after adding DCS to low pH (1.5) solution

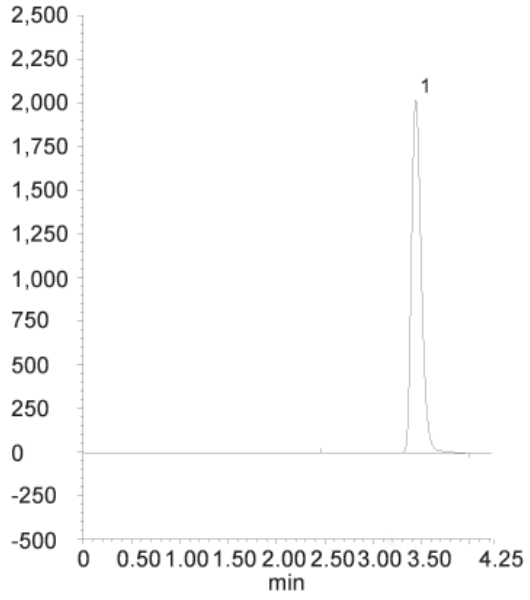

Figure 2. A representative HPLC chromatogram of D-cycloserine.

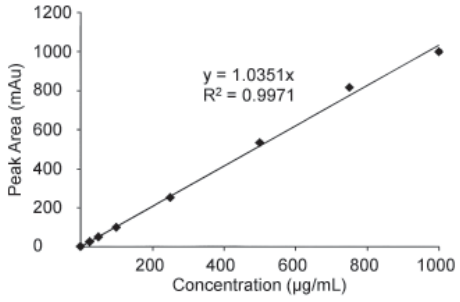

Figure 3. A standard curve for the HPLC assay of D-cycloserine.

Table 2. D-Cycloserine HPLC assay precision and accuracy (*n* = 9)

Concentration added (µg/mL)	Concentration obtained (mU/mL)	CV* (%)	RE# (%)	Recovery (%)
30	0.336	6.24	6.6	106.7
150	3.24	2.76	2.8	102.3
700	9.59	0.57	1.6	101.5

* Coefficient of Variation. # Relative Error.

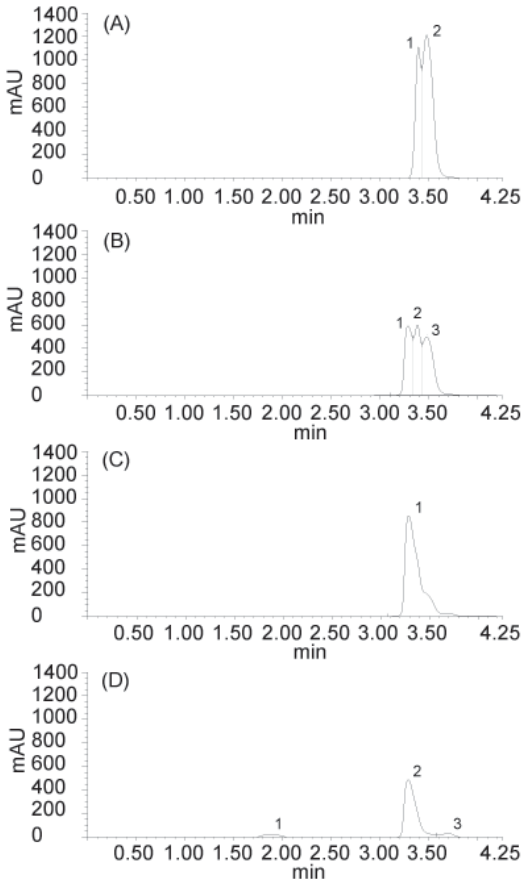

Figure 4. Chromatograms of D-cycloserine subjected to accelerated degradation at low pH of 1.5 after heating at 90°C for (A) 0 h; (B) 1 h; (C) 2 h; and (D) 4 h. The samples were analyzed by HPLC described above.

of water. As compared to the standard, the peak area of DCS decreased to around 60%, thus there was at least 40% degradation of DCS at this pH. After heating this solution at 90°C at low pH for 1 h, one more degradation product was seen on HPLC analysis (Figure 4B). In addition to the already observed degradation product 1 (deg1), observed at 3.38 min, another degradation product (deg2) was also seen at the retention time of 3.28 min. As seen in Figure 4C, after 2 h at 90°C, no peak due to deg1 was seen and only one peak of deg2 product was seen and the peak area of this product was more than double as compared to the peak area observed at 1 h (increased from around 50 mAU*min to around 140 mAU*min). After heating for 4 h, the deg2 peak decreased to half as compared to the peak area observed at 2 h (from peak area of around 140 mAU*min to around 70 mAU*min) as displayed in Figure 4D.

Under alkaline conditions, upon heating the DCS solution at 90°C, no significant change (*t*-test, $p > 0.05$) in peak area was observed for up to 3 h. Under these conditions, a reduction in peak area of DCS to around 86% was seen after 4 h. This peak further decreased to around 80% after 2 days and then to around 58% after 5 days. Standard samples were also prepared in distilled water and were subjected to high temperature of 90°C. No significant degradation (*t*-test, $p > 0.05$) was seen until 3 h. After 24 h, the peak decreased to around 60% and then further decreased to 46% after 2 days.

The DCS was observed to be light-sensitive as well because there was a decrease to around 88% of the initial concentration after 24 h when DCS was exposed to normal fluorescent light. From day one onwards, the decrease in DCS amount shows first-order degradation rate (data not shown). Thus all the DCS samples for our future studies were protected from light.

3.3. *Partition coefficient*

In our study, the partition coefficient value (Kp) determined for the DCS was logKp = –2.89 ± 0.06 ($n = 6$) for octanol against water. The log Kp value when PBS was used as an aqueous phase was –2.94 ± 0.06 ($n = 6$). LogKp and other physicochemical constants were predicted by using ACD/PhysChem software (ACD/Labs, ver. 12.01, Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2010) and the predicted values are reported in Table 1. The logKp value calculated by our studies is very close to this value. The logKp value is not very well reported in the literature and very few studies that do report this value do not cite a reference to substantiate the study carried out to determine this value. To date this is the only study where an actual analysis is done to measure the partition coefficient value and compare it with the predicted value.

3.4. *pH-solution stability*

As per the pH-solution stability profiles at different pH values (Figure 5), DCS shows extensive degradation at acidic pH values of 2.0 and 4.7. At both these pH values, the degradation was buffer ionic concentration dependent and there was a significant increase (*t*-test, $p < 0.05$) with the increase in buffer ionic concentration. At the neutral pH of 7, the degradation was not as rapid as observed at the acidic pH values. The DCS degraded to around 10% of the initial concentration in one week. No significant effect (*t*-test, $p > 0.05$) of buffer ionic concentration on the degradation of DCS was seen at the neutral pH. At slightly basic pH of 8.5, DCS shows a better stability and the effect was buffer concentration dependent as well. DCS shows significantly higher (*t*-test, $p < 0.05$) degradation at lower buffer concentration of 10 mM as compared to 20 mM. The minimum degradation of DCS was observed at the highly basic pH of 11.5. At this pH, no significant change (*t*-test, $p > 0.05$) in DCS concentration was observed over a period of 30 days and this effect was seen at both the buffer concentrations. These studies were carried out at an ambient temperature of 37°C. In our forced degradation study carried out at 90°C, the highly basic solution of DCS (pH 12.5) shows maximum stability under these adverse conditions as well.

3.5. *Reformulation of 50 mg DCS capsules*

The reformulated DCS capsules were stored under the standard conditions of room temperature (between 22 and 25°C) and three capsules samples were withdrawn

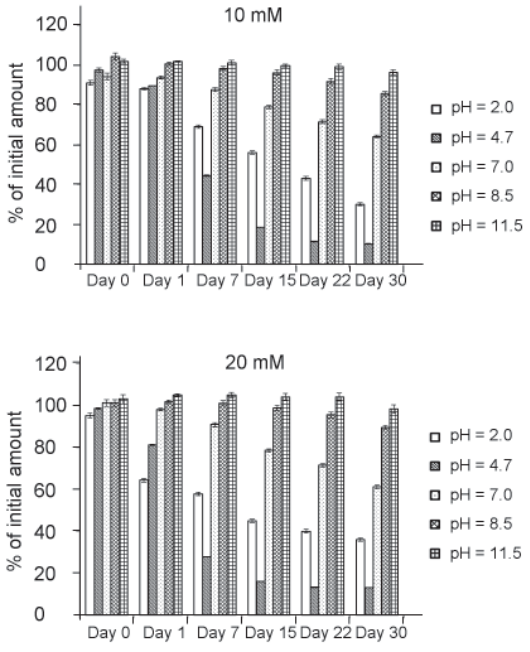

Figure 5. D-Cycloserine pH-stability profiles at 10 mM and 20 mM.

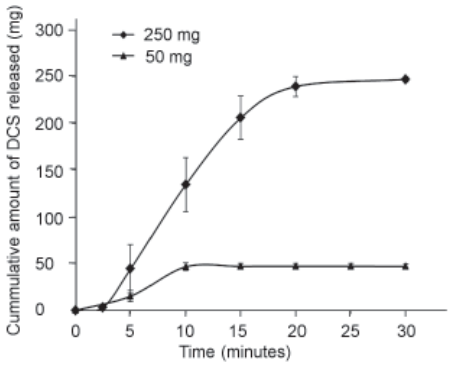

Figure 6. *In vitro* release profile of D-cycloserine from 250 and 50 mg capsules (*n* = 6).

for analysis at 0 day, 7 days, 15 days, 30 days and after every month until 12 months after this.

The weight variation test confirms to the limits as set by the USP. The percent recovery of DCS from the capsules was 100.3 ± 1.4 using this method. The stability of reformulated capsules has been done until 1 year and they contain at least 90% of the initial amount of DCS and thus are stable for at least 1 year at room temperature.

Our *in vitro* dissolution results show that all the DCS is released within 10 min from a 50 mg capsule (Figure 6). From a 250 mg capsule it takes at least 20 min for all the DCS to be completely released from the capsule.

4. Discussion

Cycloserine is degraded to serine and hydroxylamine and is relatively stable to alkali. Based on previous degradation studies, mild acid hydrolysis results in D-serine and hydroxylamine, while on prolonged hydrolysis DL-serine and hydroxylamine are formed (*15*). These were the first studies to report the hydrolysis of DCS. Prior to this, other researchers did carry out the degradation of DCS under different conditions (*15*). All these studies did observe the high stability of DCS under alkaline conditions as compared to the acidic conditions, but still the conditions that were used and the analogues that resulted were inconsistent. No study so far has used a stability-indicating HPLC method to monitor the degradation peaks.

The parent drug stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH) suggest that stress studies should be carried out on a drug to ascertain its inherent stability characteristics (*19*). A proper identification of degradation products would hence support the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability-indicating and be fully validated. In order to analyze the concentration of DCS, a stability-indicating HPLC method was validated and

Table 3. Degradation rate constants of D-cycloserine at 37°C

Buffer	pH	Order of reaction	Observed rate constant (day^{-1})	r^2
10 mM	2	First	1.9345	0.98
	4	Second	k_1 = 0.1601 k_2 = 7.567	0.99
	7	First	1.0334	0.99
	8.5	First	0.4989	0.96
	11.5	First	0.1793	0.94
20 mM	2	First	0.6508	0.93
	4	Second	k_1 = 0.1876 k_2 = 8.037	0.94
	7	First	1.3049	0.99
	8.5	First	0.401	0.91
	11.5	First	0.1987	0.95
–	SGF	First	1.2237	0.98
	SIF	First	1.6321	0.98

used for the present study. The present HPLC method meets all the acceptance criteria and was sensitive and reproducible enough for the acceptable study of DCS in unknown samples. As reported by Trissel (*20*), the failure to recognize the degradation products is the most common point that leads to erroneous reporting of the data on the stability studies. The present HPLC method does identify DCS and its degradation products based on the chromatograms of forced degradation of DCS. Thus, we can say that the present method is sufficiently specific to the drug and can simultaneously analyze DCS and its degradation products in a sample.

A drug's partition coefficient dictates the ease with which the drug reaches its intended target in the body, the potency of therapeutic response and also the transit time of the drug in the body. Thus the partition coefficient value can have a direct impact on both pharmacodynamics and pharmacokinetics of the drug. It is due to this fact that for any drug molecule, it is imperative to know this value.

DCS has good water solubility (as shown in Table 1) and based on its logKp value it can be concluded that this drug is mainly hydrophilic in character. Owing to the hydrophobic nature of the skin and its role as a barrier for keeping unwanted substances out of the body, it would be challenging to deliver a hydrophilic compound like DCS through topical route. Thus before any topical delivery system can be developed for DCS, it is very important to consider the use of an active delivery system to breach the skin barrier or other epithelial barriers, thereby allowing the drug to be absorbed in therapeutic amounts.

The degradation rate of DCS is adequately described by a pseudo first-order kinetic model for all stability samples except for pH 4. First order degradation rate constants for all the samples are included in Table 3 and have been estimated from the slopes of their corresponding log-linear plots. Extensive degradation was seen at pH value of 4 and fits into more complex second-order equation. The correlation coefficient for

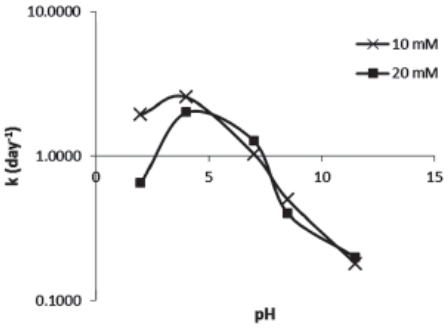

Figure 7. pH-rate profiles of D-cycloserine at 10 mM and 20 mM based on first order degradation rates.

second-order at this pH was observed to be 0.99 and 0.94 at buffer concentrations of 10 and 20 mM, respectively.

From this data, the pH of maximum stability appears to be alkaline pH of around 11.5. There was no ionic catalysis observed in the samples from buffer concentrations of 10 to 20 mM based on analysis of variance (ANOVA) at pH values of 7, 8.5, and 11.5. A probability level of $p = 0.05$ or greater indicates that no significant difference exists between the degradation rate constants estimated at 10 and 20 mM buffer concentrations. At pH of 2.0, a higher rate of degradation was observed at higher ionic strength of 20 mM as compared to 10 mM. From the plot of log K_{obs} versus pH (Figure 7), it is very clear that the reaction is primarily catalyzed by H^+ ion. DCS shows very minimal or no degradation at high pH and thus is not catalyzed by OH^- ion.

The dose of 50 mg is being used on all the clinical trials for anxiety disorders. In order to give a dose of 50 mg to the patients, the 250 mg capsule is reformulated to a 50 mg capsule. Currently, there are no pharmaceutical studies investigating the reformulation of DCS to a nominal strength. Thus, it is very important to monitor the stability of these reformulated capsules. Based on our results, these capsules do show appreciable stability at room temperature for 1 year. The *in vitro* dissolution illustrates that all the DCS is released from the capsules in 10 min. From 50 mg reformulated capsules there would be a significant loss of DCS before it can reach systemic circulation mainly because of the acidic pH in the stomach. This loss should be taken into consideration when 50 mg capsules are used through oral route. Also there is an issue of timing of administration of the current oral formulation. Using the capsules the systemic level of DCS fluctuates, which creates particular problems with the timing of administration. In some studies the DCS is administered before the session (from 30 min to 2 h), while in other studies DCS is administered after the session. Thus a formulation of DCS needs to be developed which can sustain the amount of DCS to a desired level for appreciable period of time, eliminating the conundrum of timing.

5. Conclusion

A stability-indicating method was developed, which separates all the degradation products formed. Based on the partition coefficient value –2.89, it can be established that DCS is primarily hydrophilic in character. The pH-solution stability profile shows that DCS shows maximum degradation at pH value of 4.7 and was very stable under highly alkaline conditions. The degradation was observed to be independent of ionic strength of buffer, except for pH of 2.0. The *in vitro* dissolution of these 50 mg strength DCS capsules illustrated that all the DCS is released from the capsules in 10 min. Significant degradation of DCS was observed at the acidic pH; accordingly we can conclude that there will be a significant loss of the orally administered DCS before it can reach the systemic circulation. Future directions' regarding the reformulation of this drug to maximize its efficacy should be explored.

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