Development and Validation of New RP-HPLC Method for the Determination of Dexrazoxane

M. V. BASAVESWARA RAO*, V. PRASANTHI, G. VENKATA RAO[1] AND B. V. RAMAN[2]
Department of Chemistry, Krishna University, Andhra Jateeya Kalasala Campus, Rajupeta, Machilipatnam-521 001, [1]Department of Chemistry, SRR and CVR Govt. Degree College, Machavaram, Vijayawada-520 004, [2]Department of Biotechnology, MITS School of Biotechnology, 2(P), Infocity, Patia, Chandaka Industrial Estate, Bhubaneswar-751 024, India

A new sensitive, precise, rapid and linear RP-HPLC method was developed and validated for the determination of dexrazoxane in formulations and human serum samples. Good chromatographic separation of dexrazoxane was achieved by using Kromasil C_{18} column. The system was operated at ambient temperature using a mobile phase consisting of methanol, 5% ortho phosphoric acid, 0.01M ammonium dihydrogen phosphate and tetrahydrofuran, pH 4.2 (10:40:30:20, v/v) isocratically at a flow rate of 1 ml/min. The method showed high sensitivity with good linearity (r^2=0.9998) over the tested concentration range of 0.1 to 0.9 mg/ml. Detection was carried out at 272 nm and retention time was 7.005 min. The accuracy, formulation assay and percentage of RSD were 100.03, 97.48 and 0.03184, respectively with tailing factor (1.84). This method can be used for the routine quality control analysis.

Key words: Determination, dexrazoxane, human plasma, methanol, RP-HPLC

Dexrazoxane chemically known as 4-[(2S)-2-(3,5-dioxopiperazin-1-yl) propyl] piperazine-2,6-dione (fig. 1) is a potent antineoplastic agent and catalytic inhibitor of DNA topoisomerase II[1-3]. It is the S-enantiomer of the racemic mixture of razoxane and clinically approved cardioprotective agent also called as ICRF-187 (Dexrazoxane base) with trade name Cardioxane® (Dexrazoxane hydrochloride salt), Savene® in Europe, Totect® in USA[4-7] and only approved antidote in Europe to protect the subcutaneous tissues necrosis from anthracycline used to treat acute myeloid leukemia (AML) in children and adults[8]. Moreover, it has also been identified for first time as significant protective agent against teniposide induced DNA damage and effective suppressor of apoptosis signaling process in the bone marrow cells *in vivo*[9]. It might act through its rings-opened hydrolysis product, ADR-925, which can either remove iron from the iron-doxorubicin complex or bind to free iron, thus it can be used in a broader spectrum of diseases that are known to be benefited by antioxidant treatments[10,11]. Review of literature indicates that there are several methods for the determination of dexrazoxane in human plasma and dosage forms by RP-HPLC[12-14]. But, there is no method has been reported for estimation of dexrazoxane in formulations. So, an attempt was made to develop and validate a simple, precise, accurate and economical RP-HPLC method as per ICH guidelines for the estimation of dexrazoxane in pure pharmaceutical dosage forms and to apply the developed method to determine the forced degradation compounds.

A mixture of methanol, 5% orthophosphoric acid, 0.01 M ammonium dihydrogen phosphate and tetrahydrofuran (10:40:30:20 v/v) was prepared with ultra bath sonicator for 30 min. The solution was filtered through membrane filter and degassed before use.

Stock solution of dexrazoxane was prepared by dissolving accurately weighed 500 mg of drugs in 100 ml methanol (5 mg/ml). The prepared stock solutions were stored away from light. From the

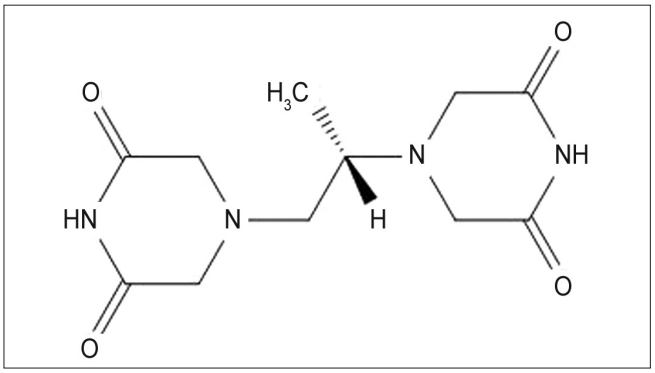

Fig. 1: Structure of dexrazoxane

***Address for correspondence**
E-mail: professormandava@gmail.com

stock, standard solutions was freshly prepared during the day of analysis. From the stock solution 3 mg/ml solution was prepared. The desired concentration for the drug was obtained by accurate dilution and the analysis was followed up as in the general analytical procedure.

Dexrazoxane in pharmaceutical dosage form was estimated by taking a standard trade form of drug in 250 mg of Zinecard strength as labeled amount. Each sample was analyzed in triplicate after extracting the drug. The amount of drug present in formulation was calculated by comparing the mean peak area from standard using the following formula. Amount of drug=(Sample peak area×dilution factor of standard×average weight of tablets)/(standard peak area×dilution factor of sample).

Twenty milligrams of formulation powder was taken from Zinecard (250 mg) and dissolved in 10 ml of mobile phase and injected into HPLC and chromatogram was recorded. The amount of drug present in the 1 mg formulation was calculated from linearity graph (figs. 2 and 3).

Blood was collected from a local hospital and serum was separated. To the 5 ml of serum, 100 µl of diltiazem hydrochloride (1 µg/ml), 0.1 ml of 1 M NaOH and 5 ml of dichloromethane were added and mixed for about 20 min in vortex mixer and centrifuged at 3000 rpm for 10 min. From this centrifuged solution 4 ml of organic layer was separated and evaporated to dryness to get residue. To this residue 100 µl of 1 M acetic acid and 3 ml of n-hexane was added and vortexed for 5 min, then the organic layer was evaporated and the remaining sample was injected into HPLC and chromatogram was recorded. The amount of drug present in the blood sample was calculated from linearity graph (fig. 4).

From linearity graph we can estimate amount of drug present in the sample. Y=mx+c, where Y=area; m=slope; x=concentration, and c=intercept. Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to give a concentration of 0.1, 0.3, 0.5, 0.7 and 0.9 mg/ml. Injection was made at intervals of 12 min. The peak area ratio of the drug was plotted against concentration. The linearity was evaluated by linear

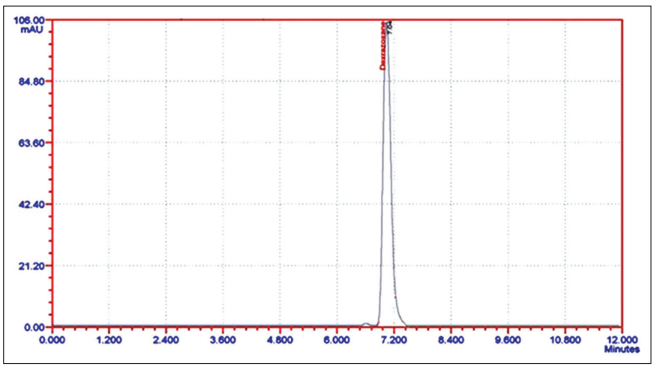

Fig. 2: A typical HPLC chromatogram of pure dexrazoxane. Retain time is 7.045, Tail Factor 1.13, Theoretical plate value is 8875

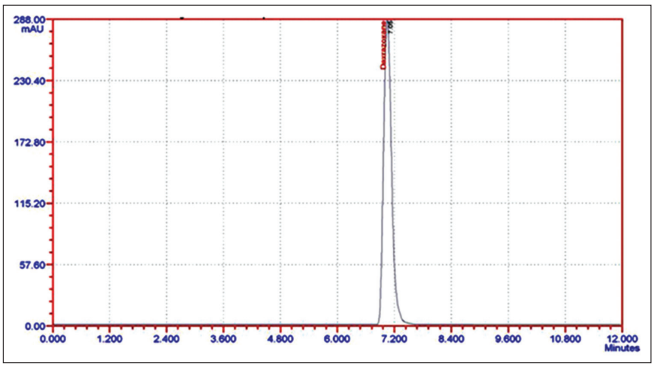

Fig. 3: An HPLC Chromatogram of dexrazoxane in sample formulations. Retain time is 7.052, Tail factor 1.16, Theoretical plate value is 6101

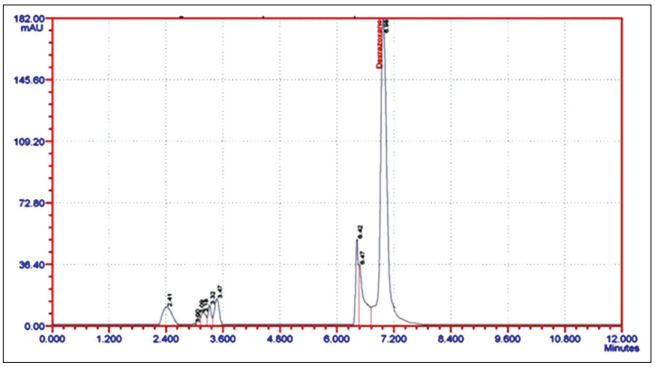

Fig. 4: An HPLC chromatogram of dexrazoxane in serum sample. Retain Time is: 6.975, Tail factor is 1.80, Theoretical plate value is 8533

regression analysis, which was calculated by the least square regression method (Table 1).

Accuracy studies were evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed. Solutions were prepared containing known amounts of dexrazoxane in the presence of excipient in the sample formulation at 80, 100 and 120% of the assay concentration. Sample solutions were prepared in triplicates for each spiked level. These solutions

TABLE 1: OPTICAL CHARACTERIZATION OF DEXRAZOXANE

Parameters	Dexrazoxane
Linearity range (mg/ml)	0.1 to 0.9
Correlation coefficient (r)	0.9998
Slope (m)	451538.45
Intercept (c)	−0.128714136
LOD	20
LOQ	60
Tailing factor (T)	1.84
Retention time (min)	7.005
Theoretical plates	5516
(%) R.S.D	0.03184
(%) Accuracy	100.03
(%) Formulation assay	97.48
Serum Concentration (mg/5 ml)	0.3

were analyzed by the HPLC method against standard at 100 and theory (Table 1)

Precision of the method was determined by assessing the repeatability (intra-day) and intermediate precision (inter-day). Repeatability was carried out by performing five repeated analysis of the same day, under the same experimental conditions. System suitability tests were carried out on freshly prepared standard stock solutions of dexrazoxane and it was calculated by determining the standard deviation of dexrazoxane by injecting standards in five replicates at 6 min interval and the values were recorded.

An UV scan of dexrazoxane showed a maximal absorbance at 272 nm. Good chromatographic separation of dexrazoxane was achieved by using Kromasil C_{18} column 250×4.6 mm with 5 µ particle size at ambient temperature with the mobile phase consisted of methanol and 5% orthophosphoric acid and 0.01 M ammonium dihydrogen phosphate and tetrahydrofuran pH 4.2 (10:40:30:20 v/v). Mobile phase filtered through 0.45 µ membrane filter was delivered isocratically at a flow rate of 1 ml/min. The injection volume was 20 µl and the total run time was 12 min (Table 1 and fig. 2). The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. A new validation method for the determination of dexrazoxane in formulations and human serum samples of patients using this drug was developed using RP-HPLC method as a stability indicating assay method (fig. 2). Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile, tetrahydrofuran (THF), and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate, orthophosphoric acid in different volume ratios. Different columns like C_8, C_{18}, phenyl, cyano with different dimensions were used. Then retention time and tailing factor were calculated. Finally methanol and 5% ortho phosphoric acid and 0.01M ammonium dihydrogen phosphate and tetrahydrofuran in the volume of ratio 10:40:30:20 v/v (pH 4.2) and Kromosil C_{18} analytical column was selected which gave a sharp and symmetrical peak with 1.84 tailing (figs. 2-4).

The linearity was tested for the concentration ranging from 0.1 to 0.9 mg/ml. The peak area ratio of the drug was plotted against concentration. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. Calibration graph was found to be linear in five different concentrations of dexrazoxane prepared at a range 0.1 to 0.9 mg/ml and 20 µl of each concentration injected to HPLC. The slope (m) and intercept (c) obtained were found to be 451538.45 and -0.128714136, respectively. The correlation of coefficient (r^2) obtained was found to be 0.9998. Results indicate that the concentration range showed a good relationship and method is linear over the concentration range studied (Table 1 and figs. 2 and 3). Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 80, 100 and 120% to the preanalyzed sample formulation. The limit of detection for dexrazoxane was found to be 20 µg/ml and the limit of quantification was found to be 60 µg/ml. It proves the sensitivity of the method. The percentage assay of dexrazoxane in formulation was found to be 100.03%. Repeatability was studied by calculated the relative standard deviation (RSD) for six determinations of the concentration of 3 mg/ml performed on the same day and under same experimental conditions. The results of dexrazoxane determinations in the working standard solution with the RSD calculated as 0.03184% are shown in Table 1. The relative standard deviation value obtained was below one which indicates the precession of the method (figs. 3 and 4). Inter-day variations were performed by using six replicate injections of standard and sample solutions of concentrations, which were prepared

and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation. Robustness was carried out by varying two parameters from the optimized chromatographic conditions**.** The method was determined as specific by comparing test results obtained from analyses of sample solution containing excuse ingredients with that of test results those obtained from standard drug.

The system suitability parameters like theoretical plates, tailing factor (T), LOD (µg/ml), LOQ (µg/ml) were calculated and compared with standard values to ascertain whether the proposed RP-HPLC method for the estimation of dexrazoxane in pharmaceutical formulations or biological samples was validated or not (Table 1). The system suitability parameters are within the specified limits and which refers the commonly used excipients and additives present in the pharmaceutical formulations did not interfere in the proposed method. From the optical characteristics of the proposed method it was found that the dexrazoxane obeys linearity range with the concentration range. The validation of the proposed method was further verified by recovery studies. The percentage recovery was found to be 97.48%, pharmaceutical formulation, Zinecard (250 mg) and its percentage assay is 100.03, which shows a good index of accuracy of the developed method (fig. 4). The amount of drug present in the human serum sample was calculated from the linearity graph was found to be 0.3 mg/5 ml.

Developed method for estimation and determination of dexrazoxane was found to be simple, precise, accurate and rapid. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested noninterference of formulation excipients in the estimation.

The reverse phase high performance liquid chromatographic (RP-HPLC) method for the analysis of dexrazoxane from their formulations was found to be accurate and precise. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of dexrazoxane formulations.

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REFERENCES

1. Karin J, Timo B, Franziska M, Hans-Joachim S. Anthracycline extravasation injuries: Management with dexrazoxane. Ther Clin Risk Manag 2009;5:361-6.
2. Langer SW, Sehested M, Jensen PB. Dexrazoxane is a potent and specific inhibitor of anthracycline induced subcutaneous lesions in mice. Ann Oncol 2001;12:405-10.
3. Hasinoff BB, Abram ME, Barnabé N, Khélifa T, Allan WP, Yalowich JC. The catalytic DNA topoisomerase II inhibitor dexrazoxane (ICRF-187) induces differentiation and apoptosis in human leukemia K562 cells. Mol Pharmacol 2001;59:453-61.
4. Rosing H, van Gijn R, ten Bokkel Huinink WW, Beijnen JH. High Performance Liquid Chromatographic Analysis of the Cardioprotective Agent Dexrazoxane in Human Plasma and Urine. J Liq Chromatogr Relat Technol 1997;20:583-601.
5. Hasinoff BB, Kellmann K, Herman EH, Ferrans VJ. Chemical, biological an clinical aspects of Dexrazoxane and other Bisdioxopiperazines. Curr Med Chem 1998;5:1-28.
6. Mouridsen HT, Langer SW, Buter J, Eidtmann H, Rosti G, de Wit M, *et al*. Treatment of anthracycline extravasation with Savene (dexrazoxane): Results from two prospective clinical multicentre studies. Ann Oncol 2007;18:546-50.
7. Schulmeister L. Extravasation management: Clinical update. Semin Oncol Nurs 2011;27:82-90.
8. Vos FY, Lesterhuis WJ, Brüggemann RJ, Graaf WT. Recovery of symptomatic extravasation of liposomal doxorubicin after dexrazoxane treatment. Anticancer Drugs 2012;23:139-40.
9. Bakheet SA, Attia SM, Al-Rasheed NM, Al-Harbi MM, Ashour AE, Korashy HM, *et al*. Salubrious effects of dexrazoxane against teniposide-induced DNA damage and programmed cell death in murine marrow cells. Mutagenesis 2011;26:533-43.
10. Junjing Z, Yan Z, Baolu Z. Scavenging effects of dexrazoxane on free radicals. J Clin Biochem Nutr 2010;47:238-45.
11. Cascales A, Sánchez-Vega B, Navarro N, Pastor-Quirante F, Corral J, Vicente V, *et al*. Clinical and genetic determinants of anthracycline-induced cardiac iron accumulation. Int J Cardiol 2012;154:282-6.
12. George L. HPLC methods for recently approved pharmaceuticals. Hoboken, New Jersey: Published by John Wiley and Sons, Inc.; 2005. p. 172-3.
13. Rosing H, ten Bokkel Huinink WW, van Gijn R, Rombouts RF, Bult A, Beijnen JH. Comparative open, randomized, cross-over bioequivalence study of two intravenous dexrazoxane formulations (Cardioxane and ICRF-187) in patients with advanced breast cancer, treated with 5-fluorouracil-doxorubicin-cyclophosphamide (FDC). Eur J Drug Metab Pharmacokinet 1999;24:69-77.
14. Brier ME, Gaylor SK, McGovren JP, Glue P, Fang A, Aronoff GR. Pharmacokinetics of dexrazoxane in subjects with impaired kidney function. J Clin Pharmacol 2011; 51:731-8.

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