Analytical Methods

Cite this: *Anal. Methods*, 2012, **4**, 1019

www.rsc.org/methods

PAPER

Quantitative estimation of DRDE-07 in mice urine using ion-paired reversed-phase high-performance liquid chromatography

Pankaj Verma,*[a] Anshoo Gautam[b] and R. Vijayaraghavan[b]

Received 20th December 2011, Accepted 7th February 2012
DOI: 10.1039/c2ay05916a

DRDE-07 is a newly synthesized amifostine analog found to be very effective as a prophylactic agent against sulphur mustard toxicity. It has been proven that orally administered DRDE-07 is more efficacious than amifostine, against percutaneously administered SM. We validated an analytical protocol for DRDE-07 in mice urine using high performance liquid chromatographic method. Heptane sulphonic acid and tetramethyl ammonium chloride were used in the mobile phase as an ion pairing agent to perform the chromatographic separation. UV detection was carried out at 249 nm, a wavelength at which an absorption peak was detected. The calibration curve for DRDE-07 was linear in the range from 1 to 100 μg ml^{-1}. The lower limit of quantification (LLOQ) was 1 μg ml^{-1}. The results demonstrate that this method has high linearity ($R^2 = 0.9986$), compound specificity, and acceptable precision/accuracy. The protocol is suitable for *in vivo* determination of DRDE-07 in mice urine.

1. Introduction

Bis(2,2′chloroethyl) thioether, commonly known as sulfur mustard (SM) is a bifunctional mustard agent and used in warfare because of its toxic effects on humans and other animals. Several reports are available of its recent use.[1–4] Simple synthesis, easy stockpiling and the toxic nature of SM make this chemical a threat worldwide and there is a high probability that terrorist groups and military opponents may use this agent during conflicts. The destructive properties of this compound, combined with the lack of effective antidote, has led some experts to classify SM as the most significant of chemical warfare agents.[5] To date no promising antidote against vesicating or other toxic actions of SM have been developed.[6–10] DRDE-07 [S-2(2-amino-ethylamino)ethyl phenyl sulphide] an analogue of amifostine was found to be very effective as a prophylactic agent against sulphur mustard toxicity. The protection offered by DRDE-07 is better than that of amifostine by the oral route. Decrease in body weight and the depletion of GSH induced by SM were significantly protected by DRDE-07. DNA damage induced by SM was also significantly reduced by amifostine and DRDE-07. Both *in vitro* and *in vivo* data indicate promising roles of DRDE-07 as a prophylactic agent against SM poisoning.[11–13] Although extensively used in the toxicology investigations, no pharmacokinetic data are available for any species that allow a direct comparison, evaluation of the effect of DRDE-07 on tissue and persistence, metabolic intermediates and excretion products under a standard set of conditions. This investigation helps fill this gap by defining pharmacokinetics in mice exposed to DRDE-07. DRDE-07 is a new molecule and hence it is essential to study its pharmacokinetics. There is no published report on either high-performance liquid chromatographic (HPLC) assay method or pharmacokinetics of DRDE-07. Therefore, an HPLC assay method has been developed and validated for the quantitative estimation of DRDE-07 in mice urine, which can be applied to study its pharmacokinetics.

2. Experimental

2.1 Chemicals and reagents

Heptane sulfonic acid (HSA) and tetramethyl ammonium chloride (TMAC) were purchased from Aldrich (USA). Acetone, ethyl acetate orthophophoric acid and potassium dihydrogen phosphate were purchased from Merck (Germany). Urine was collected from ten control animals by placing the mice in the metabolism cages for 24 h with food and water freely accessible. HPLC grade acetonitrile and methanol were obtained from J.T Baker (Philipsburg, NJ). DRDE-07 (Fig. 1) was synthesized in our synthetic chemistry

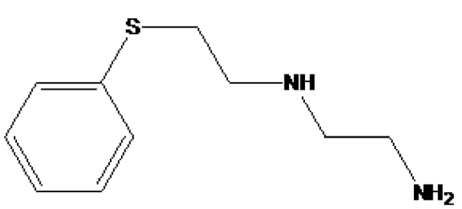

Fig. 1 Structure of DRDE-07 [S-2(2-aminoethylamino)ethyl phenyl sulphide].

[a]*Dept. of Pharmaceutics, Anand College of Pharmacy, Keetham, Agra, 282007, Uttar Pradesh, India. E-mail: pankaj4verma@hotmail.com; Fax: +91-5613202110; Tel: +91-7409069596*
[b]*Department of Pharmacology and Toxicology, Defence Research and Development Establishment, Gwalior, 474002, Madhya Pradesh, India*