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Brief Communication

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THERMOSPRAY LIQUID CHROMATOGRAPHY MASS SPECTROMETRY OF THIOL RADIOPROTECTIVE AGENTS: CHARACTERISTIC SPECTRA

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1. INTRODUCTION

Ethiofos (S-2(3-aminopropylamino)ethylphosphorothioic acid or WR-2721) is currently being evaluated in clinical radiotherapy trials (Kligerman *et al.*, 1980) because of its potential for enhancing the efficacy of radiotherapy (Kligerman *et al.*, 1980; Yuhas and Storer, 1969). For proper drug usage and pharmacological studies, it is necessary to assess the stability of the aminothiols and the presence of impurities or decomposition products. In addition, monitoring the plasma levels of WR-2721 and its metabolites should improve the therapeutic usefulness of WR-2721. Several high-performance liquid chromatography (HPLC) methodologies have been developed that are applicable for routine analysis of aminothiols and endogenous cellular thiols (Newton *et al.*, 1981; Swynnerton *et al.*, 1984). One of these methodologies, electrochemical detection, permits the simultaneous detection of the free thiol and the disulfides (Swynnerton *et al.*, 1984).

Mass spectrometry (MS) is a sensitive technique that can confirm purity and provide useful structural information. Using a thermospray interface, samples can be introduced into the mass spectrometer from a liquid chromatograph (LC) (Vestal, 1984). The interface thermally nebulizes the eluant into a high-pressure region of the mass spectrometer where a variety of soft ionization techniques may be used to ionize the analyte molecules. We have investigated the feasibility of LC coupled to MS-detection for the analysis of thiol-containing radioprotective agents, including glutathione, WR-2721, and WR-1065, the dephosphorylated sulfhydryl form of WR-2721, which has been shown to be its active radioprotective metabolite (Calabro-Jones *et al.*, 1983).

2. METHODS

The LC-MS system was an Extrel EL Mass Spectrometer (Model 400-2) coupled to a Varian 8500 syringe pump by a Vestec Thermospray interface. The HPLC solvent consisted of 0.1 M ammonium acetate at a flow rate of 2 ml/min. The thiol compounds to be analyzed were resuspended in the mobile phase buffer to a concentration of 1 µg/µl and injected either directly into the solvent stream bypassing the HPLC column (Zorbax C-8 column, 4.5 × 150 mm) or through the column before entering the thermospray interface and the mass spectrometer. The thermospray probe temperature was optimized at 225°C, and samples were analyzed using the filament-on mode. The vapor temperature was approximately 277°C. Ion spectra were obtained by scanning from 100 to 800 daltons, and the data were collected for either positive or negative ion mass spectra. The mass ranges for scanning, as well as preliminary assignment of fragment ions, were based on the expected fragment pattern and nominal molecular weights of the compounds examined.

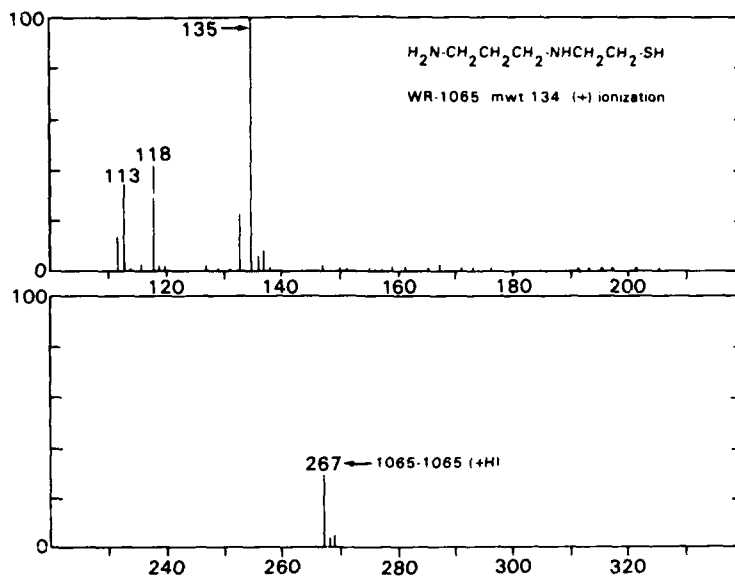


FIG. 1. WR-1065 spectra from LC-MS analyses. WR-1065 was analyzed by positive ionization techniques.

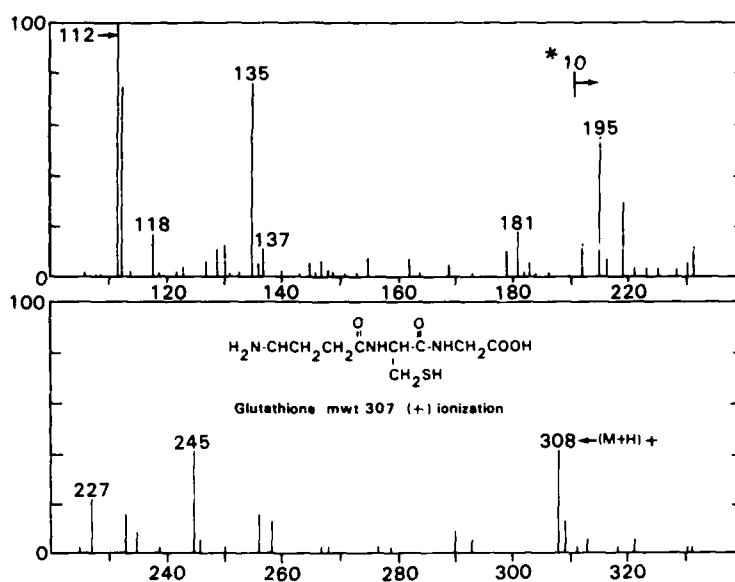


FIG. 2. Glutathione spectra from LC-MS analyses. Glutathione was analyzed using positive ionization.

3. RESULTS AND DISCUSSION

Glutathione, WR-2721, and WR-1065 were analyzed by positive chemical ionization. The positive spectra for WR-2721 and WR-1065 were indistinguishable, and only the spectrum for WR-1065 is shown (Fig. 1). The major ion occurring in the spectra of both WR-2721 and WR-1065 is at m/z 135, in agreement with an ion produced by the protonated mass of the WR-1065 mercaptan ($M+1$). A peak observed at m/z 267 is in agreement with the protonated mass of the disulfide of WR-1065. Other major peaks were observed at m/z 112 and 118. WR-2721 has a molecular weight of 214, although no $M+1$ ion was observed at m/z 215. Interestingly, the spectra for glutathione (Fig. 2) also contained an m/z ion peak at 135. Other peaks were

observed at m/z 112 and 118 as well as a small molecular ion at 308 ($M + 1$). Final identification of the chemical structures represented by the major ions will require either MS-MS analyses or the analysis of standards of probable fragments.

Preliminary experiments show that this technique can be coupled with HPLC separation to detect these thiol compounds in the extracts of plasma from mice injected with WR-2721 (data not shown). The detection response appears to be linear with the concentration of the compound, and preliminary estimates of the low sensitivity limits are near 10 pg. In addition, recent experiments (not shown) indicate that the mass spectra of WR-2721 and WR-1065 obtained with negative ionization may produce unique ions that help to distinguish the two compounds. The chromatography separation is being further refined to allow negative ionization-thermospray LC-MS analysis of both the parent compounds and their metabolites from biological samples.

Thermospray-MS appears to provide a reliable method for confirmation of thiol radioprotective agents without prior derivatization. Structural information and elemental composition about unknown thiol compounds could be obtained readily using the soft ionization MS analysis. As described here, the detection of thiols using HPLC separation and MS detection represents a novel methodology for the detection of thiols. Accurate HPLC retention times would be coupled with compound detection using selective ion monitoring of the major characteristic ion fragments. LC-MS is an exquisitely sensitive technique for detection of WR-2721 and WR-1065, with estimates for the lower limit of sensitivity in the pg range. Comparable detection limits for electrochemical methodologies for thiols (McGovern *et al.*, 1984) and for detection of fluorescent monobromobimane derivatives of thiols (Newton *et al.*, 1981) are in the low ng range.

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