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Short communication

# Thermal degradation of clozapine-N-oxide to clozapine during gas chromatographic analysis

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**Abstract**

Studies were undertaken to determine if clozapine-N-oxide, the principal urinary metabolite of the antipsychotic agent clozapine, may interfere with the gas chromatographic–mass spectrometric bioanalysis of clozapine. Following injection of clozapine-N-oxide onto a (5% phenyl)methylpolysiloxane capillary column operated at 250°C, significant on-column reduction of clozapine-N-oxide to the parent drug occurred. Accordingly, preparation of biological samples for clozapine determination by gas chromatography should avoid conditions which reportedly co-extract the N-oxide to assure no artifactual contribution of this metabolite in the detection of clozapine.

## 1. Introduction

Clozapine (Clozaril) is classified as an atypical antipsychotic agent, being clinically distinguished by exhibiting a low extrapyramidal side effect liability and by being efficacious in otherwise drug refractory schizophrenia. However, the use of clozapine has been limited due to the 1–2% incidence of drug-induced agranulocytosis [1]. The potential value of therapeutic drug monitoring of clozapine and metabolites to minimize side effects and establish a target concentration for optimal response [2,3] deserves further investigation.

This drug is extensively metabolized (Fig. 1). Gas chromatographic (GC) analysis of plasma samples from patients chronically dosed with clozapine has indicated that levels of the demethylated metabolite frequently exceed those of the parent drug [4]. Clozapine-N-oxide has been reported to be the principal [5], though less pharmacologically active [5], urinary excretion product in humans and is detectable in plasma at concentrations reported to reach 20% that of clozapine [3]. Further, the N-oxide may be metabolically reduced back to the parent compound [7]. Other urinary elimination products appear to include methiolated, hydroxylated [8], and glucuronidated [9,10] metabolites.

In the course of developing analytical methodology directed at therapeutic drug monitoring of clozapine, the present study was conducted to determine if clozapine-N-oxide potentially interferes with the GC–mass spectrometric (MS)

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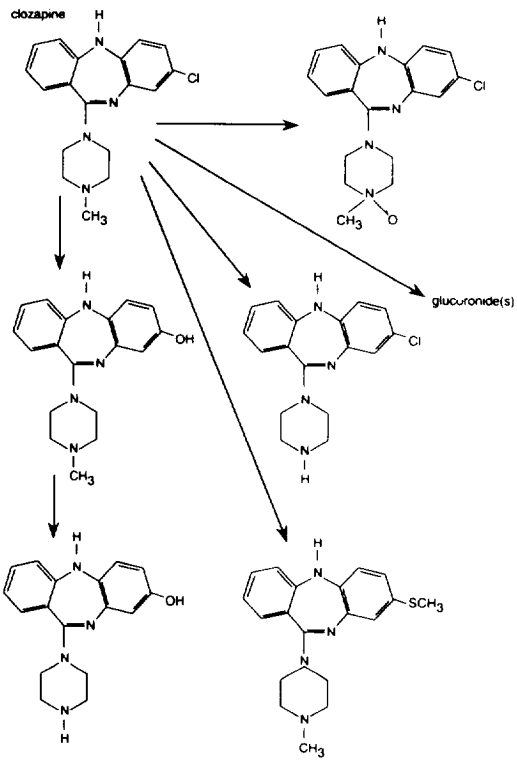


Fig. 1. Reported human metabolic pathways of clozapine.

determination of the parent drug. This is of special concern in view of the reported co-extraction of clozapine and clozapine-N-oxide from biological matrices when using liquid–liquid [6] or solid-phase [11] extractions.

## 2. Experimental

### 2.1. Materials

Clozapine and clozapine-N-oxide were obtained from Sandoz Pharmaceuticals (East Hanover, NJ, USA). Loxapine succinate was from Research Biochemicals International (Natick, MA, USA). HPLC grade methanol was from Fischer Scientific (Fairlawn, NJ, USA) and ethyl acetate was from Curtin Matheson Scientific (Houston, TX, USA). Precoated silica gel 60 thin layer chromatography (TLC) plates ($9.5 \times 4.5$ cm) were from MC/B Manufacturing Chemists (Cincinnati, OH, USA).

### 2.2. Analysis

A Finnigan Model 9610 GC-4000 MS interfaced to an IBM-AT computer and Teknivent Vector/One data system (St. Louis, MO, USA) was utilized. The injector port was adapted to capillary bore using a 17.8 cm conversion sleeve and a reducing union (Supelco, Bellefonte, PA, USA). A Hamilton 0.5-$\mu$l syringe was used to inject 0.1 $\mu$l by the splitless mode onto a (5% phenyl)methylpolysiloxane fused-silica column, 30 m $\times$ 0.32 mm I.D., 0.25 $\mu$m film thickness (DB-5, J&W Scientific, Folsom, CA, USA). The column oven and injector port were maintained at 250°C. The filament was powered 1.25 min after sample injection. Detection was by electron-impact ionization at 70 eV and 280–300 $\mu$A.

A methanolic solution of clozapine-N-oxide (3 $\mu$g/$\mu$l) was analyzed by total ion monitoring ($m/z$ 50–450) GC–MS (Fig. 2). The extent to which the N-oxide was reduced to clozapine during GC was estimated by incorporating the isosteric/isomeric internal standard loxapine (1 $\mu$g/$\mu$l), then using selected ion monitoring of the fragment ions $m/z$ 243 (base peak) for clozapine and $m/z$ 257 (59% relative abundance) for loxapine. The peak area ratio ($m/z$ 243/257) was compared against a reference clozapine (3 $\mu$g/$\mu$l)/loxapine (1 $\mu$g/$\mu$l) standard (Fig. 3).

To further investigate the conditions required for degradation of the N-oxide, methanolic clozapine-N-oxide (1 $\mu$g/$\mu$l) was heated in a

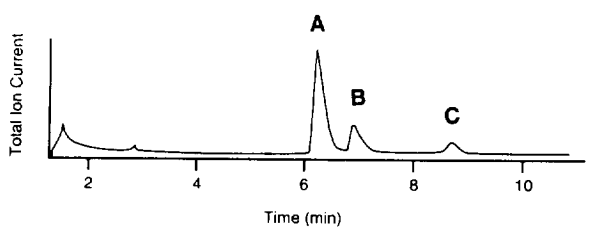


Fig. 2. GC–MS total ion chromatogram from an injection of clozapine-N-oxide demonstrating the degradation to clozapine (peak A) as based on retention time (Fig. 3) and mass spectrum (Fig. 4A).

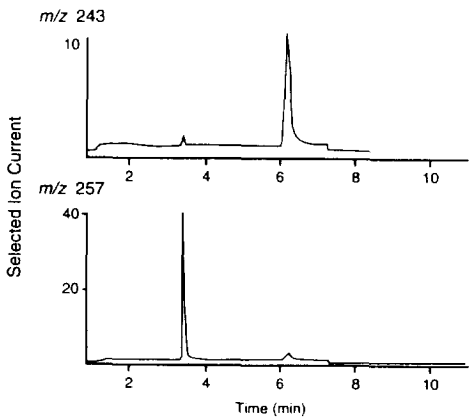


Fig. 3. Selected ion chromatograms of clozapine reference standard (above, $m/z$ 243, $t_R = 6.25$ min) and the internal standard loxapine (below, $m/z$ 257, $t_R = 3.6$ min).

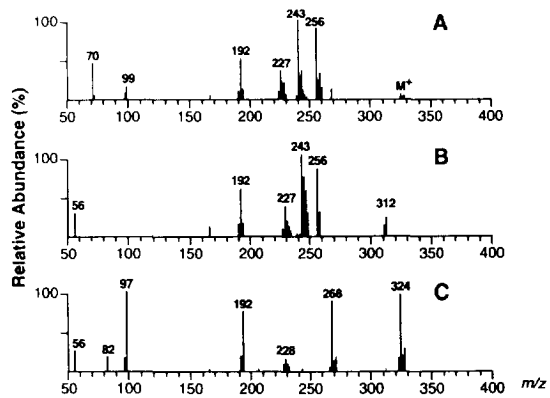


Fig. 4. Electron-impact mass spectrum of clozapine, (A) produced by GC degradation of clozapine-N-oxide. Spectra (B) and (C) correspond to the additional degradation products eluting at 6.75 and 8.55 min after injection, respectively (see Fig. 2).

glass borosilicate vial at 250°C for 30 min, then subjected to TLC along with reference standards. The TLC plates were developed using methanol–ethyl acetate (2:1) and visualized in an iodine chamber.

## 3. Results and discussion

Three components were detected after GC injection of clozapine-N-oxide (Fig. 2). The principal chromatographic peak, $t_R = 6.25$ min, was identified as clozapine based on both the retention time (Fig. 3) and mass spectrum [8] (Fig. 4A). Using selected ion monitoring of an internally standardized sample, approximately one third of clozapine-N-oxide was determined to be reduced to clozapine under the described capillary GC conditions. On-column reductions of N-oxides to the corresponding tertiary amines find a precedent in the property of other tricyclic drug N-oxides subjected to the generally more reactive conditions of packed column GC [12–14]. The TLC analysis of methanolic clozapine-N-oxide heated in a glass vial also indicated thermal degradation to clozapine (clozapine $R_F = 0.25$; clozapine-N-oxide $R_F = 0.013$).

The two GC components eluting after clozapine, peaks B and C in Fig. 2, provided the respective mass spectra of Figs. 4B and 4C. Formation of these latter components may represent thermally induced Meisenhiemer rearrangement [15] (C–N oxygen insertion) and/or enamine forming processes [13,14], both characteristic GC degradation pathways of N-oxides.

While high-performance liquid chromatography has been demonstrated to be applicable to the simultaneous bioanalysis of clozapine and clozapine-N-oxide [11], a potentially important consideration concerning sample preparation for GC analysis of clozapine is the exclusion of the N-oxide metabolite; based on the results of the present study, thermal degradation of this N-oxide to the parent drug may result in an overestimation of clozapine concentrations. Further, the TLC findings indicate that preparative measures requiring heating of samples containing the N-oxide, such as during derivatization of clozapine extracts prior to analysis, may likewise contribute to this phenomenon.

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