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Technical Note

Gas Chromatographic/Mass Spectrometric (GC/MS) Analysis of *N*-Nitrosodiisopropanolamine at the Nanogram-per-Gram Level in a Gel Formulation Containing a Nonsteroidal Antiinflammatory Agent

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INTRODUCTION

Analyses for trace levels of nitrosamines typically involve isolation of the nitrosamine from the sample matrix followed by chromatography with specific and sensitive detection. Isolation techniques must be chosen with care because of the tendency of artifacts to form, especially in the presence of the parent amine (1). Although nitrosamines are sensitive to thermal degradation, GC is usually preferred over liquid chromatography (LC) for low-level work because the detectors are generally more sensitive. A commonly used detector for nitrosamines is the thermal energy analyzer (TEA), which is very sensitive and selective for nitrosamines, although problems with false positives may arise (2). Derivatization of hydroxynitrosamines is usually performed to increase detection sensitivity, e.g., with electron-capture detection (3), or to enhance volatility and avoid thermal degradation during analysis, especially of those containing hydroxy groups. Of the silylated derivatives, the t-butyldimethylsilyl derivatives are better for GC/MS analysis because compound specific high-mass ions are present in greater abundances (4).

N-Nitrosamines have been demonstrated to be potent carcinogens in laboratory animals, even at low concentrations (5). *N*-Nitrosodiisopropanolamine (NDIPA) (CAS No. 53609-64-6) has induced pancreatic and lung tumors in hamsters and liver tumors in rats and has transformed human pancreatic cell cultures into carcinomas, but the carcinomas have not been shown to be malignant (6). Nitrosation of amines occurs readily in the presence of a nitrosating agent, such as nitrite ion in an acid environment (4). Because of these concerns, it was necessary to develop a method to measure trace levels of NDIPA in a gel formulation which contains DIPA as an excipient.

MATERIALS AND METHODS

Sample Preparation and Derivatization

Sulfamic acid (Aldrich gold label) and silica gel (E. Merck), about 0.5 g each, were mixed; then 0.5 ml water, saturated with sulfamic acid, was added and mixed. One gram of gel formulation was added, and the mixture was placed in a vacuum desiccator (0.1 Torr) for 1 hr. Following desiccation, the powder was transferred to a disposable polyethylene column, 10 cm × 1-cm I.D. Four 5-ml portions of ethyl acetate (all solvents Baker HPLC grade) were used to elute the column. Slight air pressure was used to force the solvent through the column. The eluate was collected and solvent was removed using a rotary evaporator (45°C bath, 60 Torr).

Flash chromatography (7) was performed using 25-cm × 1.45-cm-I.D. glass columns with 250-ml reservoirs and Teflon stopcocks (Kontes) slurry-packed with 12.5 g silica gel in acetonitrile. The slurry was contained in the column with a layer of sand on silanized glass wool. A layer of sand was also placed at the head of the column, and the solvent level reduced to the top of the sand. The sample was quantitatively transferred to the column using five 0.5-ml portions of acetonitrile, then eluted with acetonitrile. The first 20 ml was discarded, then the next 40 ml collected and the solvent removed as above for ethyl acetate. The residue was transferred with five 0.5-ml portions of acetonitrile to a conical 3-ml screw-cap reaction vial. The vial was placed in a 70°C heating block, and the solvent was evaporated under a stream of argon.

Seventy microliters of acetonitrile and 30 μl of silylation reagent (1 m*M* t-butyldimethylchlorosilane with 2.5 m*M* imidazole in dimethylformamide from Alltech/Applied Science) were added to the vial, which was then tightly capped (Teflon-lined septum), mixed on a vortex mixer, and heated at

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70°C for 30 min, with additional mixing after 15 min. This formed the bis (t-butyldimethylsilyl) ether.

Spiked samples were prepared by adding the appropriate volume (100 µl-maximum) of standard solutions of NDIPA (Sigma Chemical Co.) in acetonitrile to the sample prior to desiccation. System blanks and single components (e.g., DIPA) of the formulation were prepared as well. Analytical standards were prepared by pipetting the appropriate volume of standard solutions into reaction vials, evaporating the solvent under argon, and silylating as above.

Instrumental Analysis

Gas chromatography/mass spectrometry analyses were performed using a Hewlett Packard 5890 GC interfaced to a FinniganMAT 710 ion trap detector. The GC column was a 30-m × 0.25-mm-I.D. fused silica capillary coated with 0.25-mm DB5 (J&W Scientific). The oven was maintained at 60°C for 0.5 min, then increased at 30°/min to 200°C, held for 10 min, and increased again at 30°C/min to 250°C. Total GC run time was 22 min. The injector was set at 180°C. The injection volume was 3.0 µl, using 1.0 µl solvent flush. The injector was used in the splitless mode with the purge off for 0.5 min. The open-split interface was maintained at 200°C with 100% transfer. The MS was operated in EI mode monitoring the *m/z* 333 ion $[M\text{-}57]^+$ with a 10-min delay to bypass the solvent front. Areas of the derivatized NDIPA peaks were measured using the MS data system (IBM PC AT). Chromatograms of a standard (equivalent to 10 ng/g) and a gel sample (Batch A) are shown in Figs. 1a and b. The concentration of NDIPA in the gel formulation was calculated as follows:

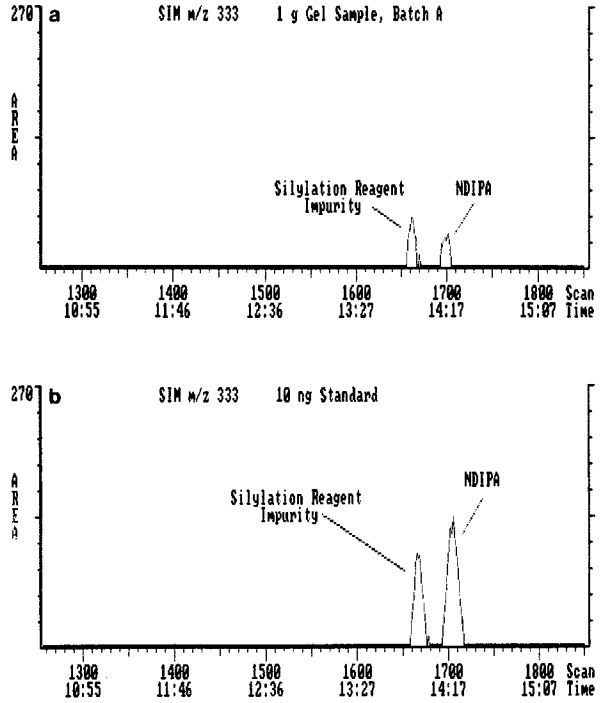

Fig. 1. Selected ion monitor (*m/z* 333, $[M\text{-}57]^+$). Chromatograms of (a) standard equivalent to 10 ng/g NDIPA in gel and (b) gel sample, Batch A.

$$K_f = \frac{\text{area of standard (average of 3 injections)}}{\text{concentration of standard (ng/100 µl)}} \quad (1)$$

$$\text{ng/g NDIPA} = \frac{(\text{area of sample}) \times (100\ \mu\text{l})}{(K_f) \times (\text{g sample weight})} \times \frac{1}{\text{recovery}} \quad (2)$$

Statistical Analysis

The chromatographic precision was determined by injecting a derivatized standard NDIPA solution, corresponding to 10 ng/g, four times. The coefficient of variation was ±4.1%. The method precision, determined by four preparations of the same simple, each injected in duplicate, was cv 11.6%. To determine recovery for this analytical procedure, the method of standard additions was used. Seventeen gel samples were spiked with NDIPA in the range of 0 to 20 ng/g and then analyzed for NDIPA content. Recovery is estimated as the slope of the linear regression for NDIPA found vs NDIPA added, as shown in Fig. 2. The recovery was 76 ± 9% at the 95% confidence level.

RESULTS AND DISCUSSION

Instrument response was found to be linear ($r = 0.9986$) for NDIPA concentrations corresponding to from 5 to 60 ng/g in the gel (Fig. 2). Higher concentrations were not investigated. The limit of detection (S/N = 3:1) corresponded to 1 ng/g or 40 pg NDIPA injected.

Three batches of the gel were prepared and analyzed. Each replicate preparation was injected in duplicate. Measured concentrations of NDIPA ranged from 2 to 4 ng/g, corrected for recovery, as shown in Table I. These would be reported as detectable but less than 5 ng/g since the values were less than the lower bound of the linear region of the calibration curve.

The method of standard additions was used to determine the recovery because a suitable internal standard was

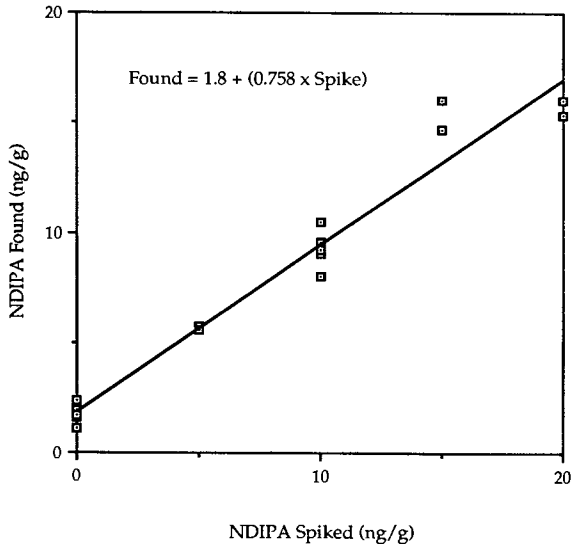

Fig. 2. Standard addition of NDIPA to gel. Slope is recovery: 76 ± 9% at the 95% confidence level.

Table I. NDIPA Concentrations in Three Gel Formulation Batches

Batch	Replicates	NDIPA concentration (ng/g)		
		Measured	Corrected	Reported
A	4	3	4	<5
B	2	3	4	<5
C	2	2	2	<5

not available. The use of *N*-nitrosodiethanolamine was explored, but it exhibited different retention characteristics during the cleanup steps. A ^{15}N isotopic label introduced in the nitroso group of NDIPA was considered, but the ^{13}C contribution from native NDIPA to *m/z* 334 would have interfered. Perdeuterated NDIPA, with its greater mass difference, might have been useful, but it was not available.

The method developed was found to be both selective and sensitive for NDIPA. Removal of DIPA in the first cleanup step and the presence of a nitrite scavenger (sulfamic acid) solved the problem of the formation of NDIPA as an artifact. Samples prepared without sulfamic acid showed elevated NDIPA levels. Other artifacts from exogenous amines did not interfere since the detection scheme was selective for NDIPA.

In the second cleanup step, the first 20 ml was discarded because it contained a component of the formulation that had a hydroxy functional group which would compete with NDIPA for the derivatizing reagent. Minimizing the amount of reagent was important since there was no postcolumn split in the GC, and all of the reagents entered the MS source. The derivatization was performed in acetonitrile for two reasons: first to improve the solvent effect in the GC and second to enhance derivatization. Various ratios of solvent to reagent were tried, and 70:30 proved optimal. Initially, a more polar GC column (DB-1701) was employed, but the diastereomer separation described by Issenberg *et al.* (4) was observed.

Occasionally, an impurity in the derivatization reagent with a minor fragment ion at *m/z* 333 was observed, as shown in Figs. 1a and b, but it eluted separately from the NDIPA silyl ether with the high resolution of the capillary column and did not interfere with the analysis. With the lower resolution of a packed column, the potential for interference is greater.

This method can be modified to accommodate different sample matrices or other target hydroxynitrosamines. However, different elution volumes or solvents during the cleanup steps may have to be employed since the retention characteristics of various hydroxynitrosamines, such as *N*-nitrosodiethanolamine (as described above), may differ. Of course, the appropriate ion must be monitored by MS.

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