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Direct Injection Gas Chromatographic/Mass Spectrometric Analysis for Denatonium Benzoate in Specific Denatured Alcohol Formulations

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Direct injection GC/MS was investigated for the analysis of benzyldiethyl(2,6-xylylcarbamoylmethyl)ammonium benzoate (Bitrex), a quaternary ammonium salt, in various Canadian denatured alcohol formulations. Bitrex yielded predominantly a peak due to the neutral diethylamine derivative (I). The structure of I, elucidated by MS and NMR, is strongly related to that of the cation of Bitrex. Compound I was formed from Bitrex in the heated injector port of the GC via a decomposition reaction similar to Stevens rearrangement. The response of I was found to be dependent on the injector port temperature, and the optimal temperature was determined to be in the range 250−350 °C. The GC/MS response of I in SIM mode was used to quantify Bitrex. The effects of the codenaturants sucrose octaacetate (SOA), diethyl phthalate (DEP), and camphor, which are present at much higher concentration than Bitrex in several formulations, were also investigated. The presence of SOA enhanced the response of the analyte considerably, while DEP and camphor had no significant effect. All standard curves of Bitrex (1−16 ppm) in different alcohol matrixes were fitted by second-order polynomial functions, with coefficients of determination (R^2) routinely in the range 0.998−0.999. The analysis time was 18 min, and the within-run precision was <4%. The results of this study point to the potential of the GC/MS technique as a quantitative tool for Bitrex in various alcohol formulations.

Bitrex, chemically known as benzyldiethyl(2,6-xylylcarbamoylmethyl)ammonium benzoate, is a highly bitter compound. It is among the common substances used for denaturing alcohol. The Canadian Government Regulations specify the level of Bitrex in several formulations of specially denatured alcohol to be 7 ppm. The rate of duty on the denatured alcohols depends on whether the specifications are met. Various methods based on thin-layer chromatography and high-performance liquid chromatography have been applied to the determination of Bitrex in different matrixes, such as alcohol toilet preparations,[1,2] rapeseed oils[3] and denatured alcohols.[4] All these methods involve preconcentration of Bitrex prior to chromatographic analysis. They are time-consuming, require a large sample volume, and may suffer errors inherent to the extraction process. Therefore, a direct approach which does not involve any sample preparation for the determination of Bitrex in alcohol formulations is highly desirable.

Quaternary ammonium salts are thermally unstable. They decompose at elevated temperatures to neutral molecules which could be vaporized and subsequently analyzed by various separation techniques. Gas chromatography is an ideal analytical technique for the determination of quaternary ammonium salts because the heated injector port of the instrument allows in situ decomposition of the salts. As such, transformation to neutral molecules, vaporization, and analysis of the characteristic pyrolysates can be performed in a single step. Direct injector pyrolysis has been previously applied to the analysis of quaternary ammonium salts.[5−7] For example, benzalkonium chlorides were thermally degraded to alkyldimethylamines upon injection into a gas chromatograph, and the pyrolysis products were used to determine the homologous composition of the salt. Similarly, Bitrex could be assayed by analyzing the nitrogen-containing pyrolysates. In several Canadian formulations containing Bitrex, other denaturants such as diethyl phthalate (DEP), camphor, and sucrose octaacetate (SOA) can also be present. The concentration levels of these components are usually 30−400 times higher than that of Bitrex. Trace analysis of Bitrex in the presence of large amounts of other denaturants requires a highly selective and sensitive detection technique.

The objective of this study was to show that direct injection GC/MS operated in a selected ion monitoring mode (SIM) is a feasible approach for the determination of Bitrex in various alcohol formulations. Quantification of Bitrex was based on the determination of a rearranged pyrolysis product (**I**). The determination of the structure of **I** by MS and NMR spectroscopy, and the analytical characteristics of the technique, are described.

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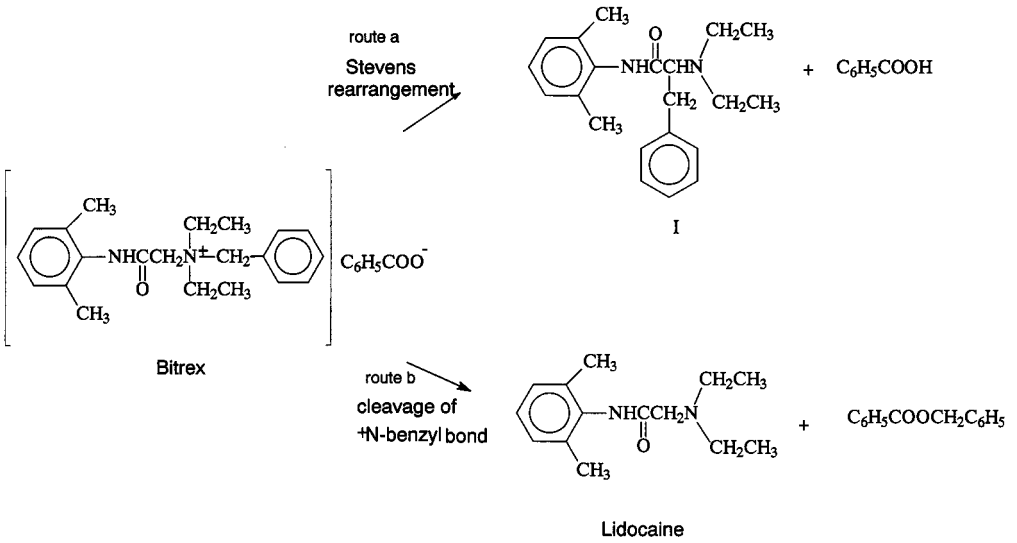

Figure 1. Major pyrolysis pathways of Bitrex in the GC injector port.

EXPERIMENTAL SECTION

Chemicals. Bitrex (98%), ethyl stearate (99%), diethyl phthalate (99%), sucrose octaacetate (99%), and camphor (96%) were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received, and 95% (v/v) ethyl alcohol was supplied by Les Alcools de Commerce Co. (Quebec, Canada).

Gas Chromatography/Mass Spectrometry Analysis. Quantification was carried out using a Hewlett-Packard GC/MSD model 6890 system. The system included a 6890 GC with electronic pressure control, the MSD, an autosampler, and a Chemstation. The analytical column was a DB5ms (J&W Scientific, Folsom, CA), 30 m long, 0.25 mm i.d., and 0.5 μm film thickness. Helium was the carrier gas at a constant flow of 1.1 mL/min. A 1-μL aliquot of the alcoholic solution was introduced into the GC/MS system in the splitless mode, and the injector was purged after 1 min. The injector glass sleeve was a 4-mm-i.d. borosilicate single-taper type liner with a small glass wool plug at the tapered end. The temperature program was as follows: initial 85 °C held for 1 min; 30 °C/min to 300 °C, held for 9.83 min. The total run time was 18 min. The injector and interface temperatures are 275 and 290 °C, respectively. The mass-selective detector was operated in the SIM mode, with a solvent delay of 8 min. Two groups of ions, m/z 176 and 233, and m/z 88 and 101, were used to monitor Bitrex and ethyl stearate, the internal standard (ISTD), respectively. The dwell time for each ion was 150 ms. The ions at m/z 176 and 88 were used for quantifying Bitrex and ISTD, respectively.

Qualitative analysis was also performed on a Varian Saturn III GC/MS system which was equipped with a Star 3400CX GC, an 8200 CX autosampler, an Alltech EPC 1000 electronic pressure control system, the Saturn III Ion Trap, and a Chemstation. GC column and conditions were the same as described above. The trap was kept at 280 °C and was scanned from 40 to 500 amu at a rate of 1 scan/s.

Nuclear Magnetic Resonance Analysis. ^{1}H and ^{13}C NMR spectra of **I** and Bitrex were recorded at 25 °C on a JEOL 270 spectrometer operating at 270 MHz and using $CDCl_3$ as solvent. The ^{1}H NMR spectra were collected in 16 384 data points over a 4.052-kHz spectral width, with a 45° pulse width of 5 μs and a relaxation delay of 1 s. The ^{13}C NMR spectrum of **I** was acquired in 32 768 data points over a spectral width of 16.92 kHz, with a 90° pulse width of 10 μs and a relaxation delay of 5 s. A double-quantum-filtered COSY 2D NMR spectrum was collected in a 1024 × 512 matrix with a spectral width of 2.026 kHz.

Preparation of Compound I. About 35 mg of Bitrex was placed in a Pyrex tube, 4 mm i.d. and 75 mm long. Glass wool was introduced from both ends of the tube to confine Bitrex to a 5-mm section of the tube. A small heating block at 250 °C was mounted over the tube where Bitrex was contained while N_2 was being passed through the tube at 30 mL/min. The volatile products of the pyrolysis, such as benzoic acid, benzyl benzoate, and lidocaine, were flushed away using a N_2 stream. The less volatile components were dissolved in ~2 mL of CH_2Cl_2, and the solution was concentrated to ~100 μL under N_2 before being added to a SPE C-18 cartridge (Waters, MA). Compound **I** was eluted using ~2 mL of CH_2Cl_2. The eluent was evaporated to dryness under N_2 and redissolved in deuterated $CHCl_3$ for GC/MS and NMR analysis.

RESULTS AND DISCUSSIONS

Structure of the Major Pyrolysis Product. Quaternary ammonium compounds are known to decompose thermally by cleavage of a C−N bond at the quaternary nitrogen with the formation of a tertiary amine and another neutral compound.[5,7] In general, debenzylation is favored over dealkylation (Figure 1, route b). In this work, in addition to the expected pyrolysis products, lidocaine and benzyl benzoate, the thermal decomposition of Bitrex in the GC injection port gave a major product with a retention time of 10.25 min (Figure 2) corresponding to the diethylamine derivative **I**, and benzoic acid (Figure 1, route a).

Bitrex contains a strong electron-withdrawing 2,6-xylycarbamoyl group attached to one of the methylene carbons around the quaternary nitrogen atom. Compounds of similar structure have been reported to undergo Stevens rearrangement in the presence of a base to give rearranged tertiary amines.[8] The reaction involves loss of a labile proton from the methylene group adjacent

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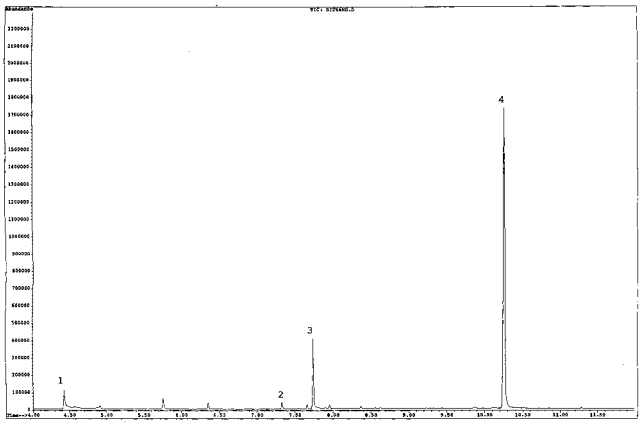

Figure 2. Total ion chromatogram resulting from injection of an alcohol solution of Bitrex into GC/MS in full-scan mode: (1) benzoic acid, (2) benzyl benzoate, (3) lidocaine, and (4) compound **I**.

to the 2,6-xylylcarbamoyl moiety with subsequent migration of a substituent on the quaternary nitrogen. The formation of **I** and benzoic acid from Bitrex in the heated GC injector port can be explained via a similar rearrangement. In this case, the benzoate ion serves as the base, and the benzyl substituent is the migrating group.

The diethylamine derivative **I** was characterized by MS and NMR. The EI mass spectrum of **I** obtained from a quadrupole mass filter shows a base peak at m/z 176, with small peaks at m/z 148 and 233, while the molecular ion (m/z 324) was not observed (Figure 3a). The spectrum obtained from ion trap spectrometry is very similar to that in Figure 3a, except that, in addition, a distinct peak at m/z 325 was detected (Figure 3b). This is the $[M + 1]^+$ ion which is often formed in ion storage mass spectrometry.[9] This experiment provides evidence that **I** has a molecular weight of 324. The fragments at m/z 176 and 233 are ions resulting from α-cleavage with loss of 2,6-xylycarbamoyl and benzyl groups, respectively, when the tertiary nitrogen is the charge-initiation site. α-Cleavage at the carbonyl group yields the peak at m/z 148 when **I** is ionized at the carbonyl oxygen.

Compound **I** had the same retention time and mass spectrum as the major peak in the total ion chromatogram obtained from direct injection of Bitrex, but its ^{1}H NMR spectrum (Figure 4) was entirely different from that of Bitrex. The assignments of peaks are shown in Table 1. The methine proton (a) and the benzylic protons (b and c) constitute a spin system. The two benzylic protons are not chemical shift equivalent since they are attached to an asymmetric carbon. Each proton is split by the other and by the neighboring methine proton, resulting in a four-peak pattern. The methine proton is coupled almost equally to both benzylic protons ($J_{ab} = 6.6$ Hz, $J_{ac} = 5.6$ Hz) and, therefore, appears as a triplet (doublet of doublet) at δ 3.84. The methylene protons (d and e) on the ethyl groups appear as strongly coupled spin systems at δ 2.54 and δ 2.72. This is in agreement with previously reported nonequivalence of the methylene protons,

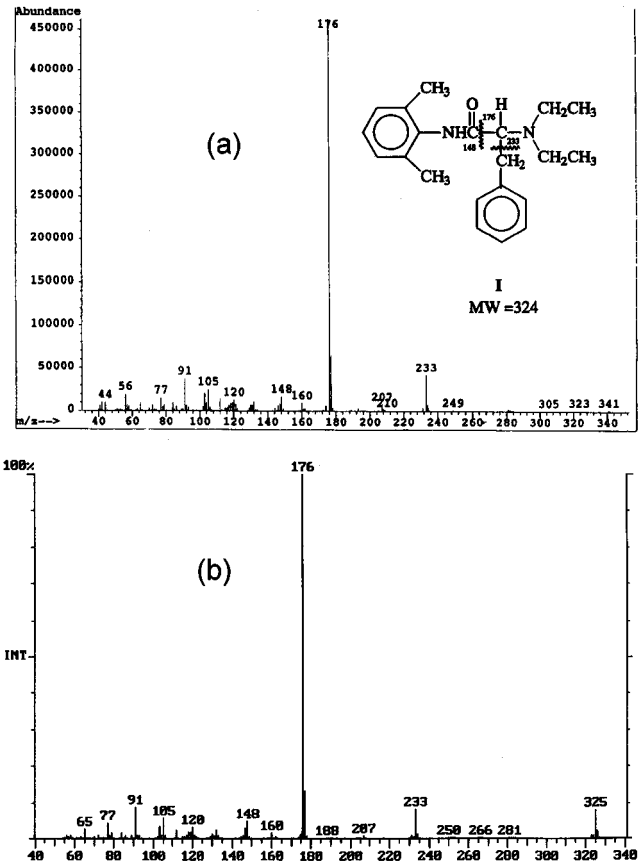

Figure 3. Mass spectra of compound **I** obtained from (a) quadrupole mass spectrometer and (b) ion-trap mass spectrometer.

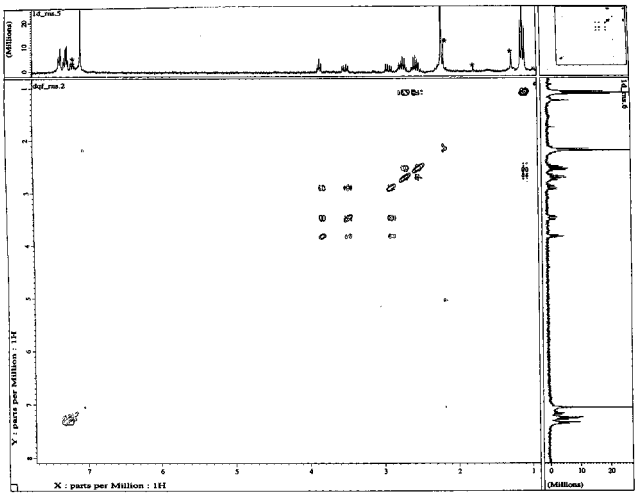

Figure 4. COSY NMR of compound **I**. Peaks marked with an asterisk are due to impurities.

even if they are more than one atom away from the chiral center.[10] The shift difference between the two methylene protons is small (48.6 Hz) compared with a geminal coupling constant of 13.4 Hz. Consequently, the AB pattern from the geminal coupling is quite distorted; the inner peaks are strong, while the outer peaks are weak. Each methylene proton is also split by the methyl protons at δ 1.1 with slightly different coupling constants, 7.0 and 6.8 Hz. Some of the peaks coincide, giving rise to six peaks. The singlet at δ 7.05 is assigned to the aromatic protons of the 2,6-xylycarbamoyl group, since the three aromatic protons of lidocaine,

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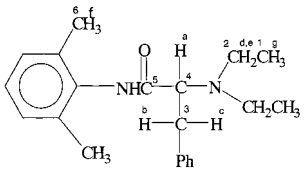
Table 1. NMR Data for Compound I



proton (type, no of protons)	chemical shift δ, ppm (multiplicity)	coupling constant J, Hz
a (methine, 1)	3.84 (d of d)	$J_{ac} = 6.6$, $J_{ab} = 5.6$
b (methylene, 1)	3.48 (d of d)	$J_{ab} = 5.6$, $J_{bc} = 13.7$
c (methylene, 1)	2.91 (d of d)	$J_{ac} = 6.6$, $J_{bc} = 13.7$
d,e (methylene, 4)	2.54 (m), 2.72 (m)	$J_{de} = 13.4$, $J_{gd} = 7.0$, $J_{ge} = 6.8$
f (methyl, 6)	2.2 (s)	
g (methyl, 6)	1.1 (t)	$J_{gd} = 7.0$, $J_{ge} = 6.8$
aromatic (2,6-xylylcarbamoyl, 3)	7.05 (s)	
aromatic (benzylic, 5)	7.2−7.4 (m)	

carbon-13 (type)	chemical shift δ, ppm
1 (methyl)	14.3
2 (methylene)	45
3 (methylene)	31.5
4 (methine)	66
5 (carbonyl)	172
6 (methyl)	18.6
aromatic (2,6-xylylcarbamoyl)	127−133.5
aromatic (benzylic)	128−130

which contains the same aromatic moiety, also appear as a singlet at δ 7.07. The COSY spectrum (Figure 4) confirms the connectivity of the coupled nuclei described above. Peak areas of the absorptions of the proton nuclei are in agreement with their relative abundances. The chemical shifts of carbon-13 nuclei, shown in Table 1, are also consistent with the structure.

Analytical Characteristics. Compound **I** was used for quantification of Bitrex because it had a structure highly related to Bitrex, and it gave the strongest GC/MS response of all the products of pyrolysis. Since **I** was formed by heat-induced rearrangement/decomposition of Bitrex in the injector, the response may vary considerably with the injector temperature. The effect of injector port temperature over the range 200−360 °C was investigated, using a 7 ppm solution of Bitrex in alcohol. The analysis was carried out in SIM mode, and the ion at m/z 176 was used for quantification. At each temperature, four replicates were analyzed. The average response of **I** rose sharply from 200 to 250 °C and leveled off thereafter. In all the studies described below, the injector temperature was set at 275 °C. The replicates of the same sample were run nonconsecutively. Ethyl stearate, the internal standard (ISTD), was added to each test solution at a concentration of 6 ppm. A stable isotope-labeled Bitrex would be an ideal ISTD; it was, however, not used in this study because of the unavailability of the labeled compound.

The precision was determined by performing five replicate analyses of each of the three alcohol solutions containing 7 ppm of Bitrex in different matrixes. The relative standard deviations (RSDs) of the measurements of the response ratio (**I**/ISTD) were typically <4%. In all cases, no significant carryover of the analyte and the ISTD was observed in the blanks that were run after the Bitrex solutions.

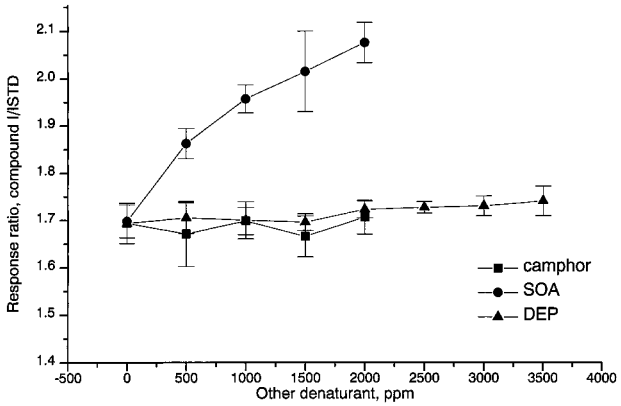


Figure 5. Effects of matrix on the GC/MS response of Bitrex.

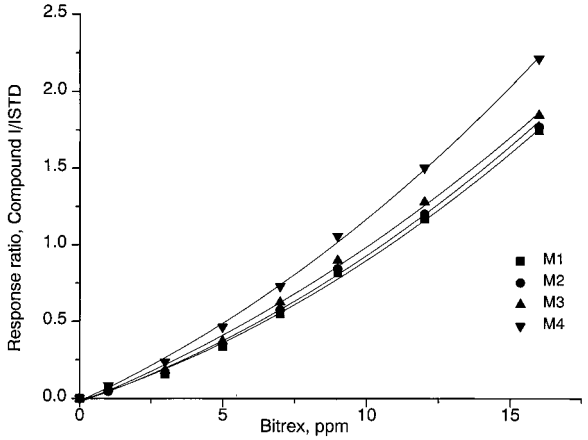


Figure 6. Standard curves of Bitrex in different matrixes. M1, absence of codenaturants; M2, 1000 ppm camphor; M3, 210 ppm SOA, 400 ppm camphor, and 2800 ppm DEP; and M4, 980 ppm SOA.

Several Canadian alcohol formulations also contain other substances as denaturants in addition to Bitrex. These codenaturants, camphor, DEP, and SOA, which eluted at 4.6, 6.6, and 14.0 min, respectively, were well separated from **I** at 10.25 min and the ISTD at 8.8 min. Although they did not interfere with peak integration, they might have an effect on the quantification of Bitrex. The possible matrix effects of camphor, DEP, and SOA on the response of **I** were determined using a series of standard alcohol solutions containing 16 ppm of Bitrex and one of the other denaturants in varying concentrations covering the typical range found in Canadian denatured alcohol formulations. As shown clearly in Figure 5, the response ratio of **I** to ISTD was not significantly affected by the presence of camphor (500−2000 ppm) and DEP (500−3500 ppm), as confirmed by ANOVA tests ($P >$ 0.1). A Bitrex solution containing 2000 ppm of camphor and 3500 ppm of DEP also gave similar results. However, the response ratio of **I** to the ISTD increased considerably with the concentration of SOA (500−2000 ppm). SOA was analyzed separately, and the results confirmed the absence of any impurity that coeluted with **I** and would interfere with the integration.

The effects of SOA were further illustrated by the standard curves of Bitrex (Figure 6). Seven Bitrex standard solutions, covering the range 1−16.3 ppm, in typical denatured alcohol matrixes of Canadian formulations—M1 (absence of any other denaturants), M2 (1000 ppm camphor), M3 (210 ppm SOA, 400 ppm camphor, and 2800 ppm DEP), and M4 (980 ppm SOA)—

and matrix blanks were analyzed in triplicates. The averaged response ratios of **I**/ISTD were plotted against Bitrex concentration. The standard curves, when fitted with linear regression, yielded *y*-residuals which showed a marked trend. However, second-order polynomial functions provided satisfactory fits. The coefficients of determination, R^2, were invariably in the range 0.998−0.999, and the intercepts were $<\pm 4\%$ of the response of Bitrex at a concentration of 7 ppm. The 95% confidence bands of the standard curves of matrixes M3 and M4 did not enclose the standard curve of M1. These results are expected because SOA was present in both M3 and M4, while M1 contained only Bitrex. On the other hand, the fitted curves of M1 and M2 were not significantly different since SOA was not present in M2. In all cases, the area counts of ISTD did not vary significantly ($P > 0.05$) across the calibration solutions; therefore, any change in response ratio was related to the response of **I**. In light of these findings, for accurate prediction of Bitrex in a sample containing SOA, it is necessary to match the matrix of the calibration solutions with that of the sample. The nonlinearity of the standard curves indicates that the response factor of **I** increased with the concentration of Bitrex. It is possible that the decomposition of Bitrex in the injector port is an intermolecular process. The mechanism of pyrolysis is presently under investigation.

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The nonlinear data can, however, approximate a linear fit if the range is suitably small. For the most accurate measurements,[11] the point corresponding to the specified level of 7 ppm is bracketed by closely spaced calibration standards. Measurements in the range of 5.6−8.4 ppm of Bitrex were practically linear ($R^2 = 0.999$), although the line did not pass through the origin ($P = 0.001$). The 95% confidence interval of Bitrex at 7 ppm was $\pm 1.5\%$ of the concentration.

CONCLUSIONS

Direct injection GC/MS (SIM) has been demonstrated to be a feasible technique for analyzing Bitrex in various alcohol formulations. The predominant pyrolysate, **I**, has a structure highly related to Bitrex and can serve as an analyte representative of Bitrex. The valuable feature of this approach remains the ability of GC/MS to quantify Bitrex in the presence of large amounts of other denaturants, such as camphor, DEP, and SOA, in alcohol solutions without any sample preparation.

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