Pyrrolizidine Alkaloid Content in Crude and Processed Borage Oil from Different Processing Stages

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ABSTRACT: The pyrrolizidine alkaloid content of crude borage oil and borage oil from different processing stages was determined by GC–MS. The results showed that no pyrrolizidine alkaloids were present above a detection limit of 20 ppb. The reduction factors for pyrrolizidine alkaloids at various stages in the oil refining process were determined by means of spiking experiments using the commercially available pyrrolizidine alkaloid crotaline. It was shown that the pyrrolizidine content in crude borage oil was reduced overall by a factor of about 30,000 in the refining process.

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KEY WORDS: Borage oil, pyrrolizidine alkaloids, GC–MS.

Pyrrolizidine alkaloids are found as natural constituents in the plant *Borago officinalis* (1–3). Most of the pyrrolizidine alkaloids reported, i.e., amabiline, supinine, lycopsamine, intermedine, and acetylated forms of lycopsamine and intermedine, have a 1,2-unsaturation in the ring structure indicating hepatotoxicity (3), but the presence of a saturated pyrrolizidine alkaloid, thesinine, also has been shown in this plant (1). The chemical structures of some of these compounds are shown in Figure 1.

Interest in using borage oil as a source of γ-linolenic acid has increased during the last few years in both the pharmaceutical and health food industries. It is used in parenteral lipid emulsions, in skin formulas, and as a dietary supplement. The oil, extracted from the seeds of *B. officinalis*, contains about 20% (w/w) of γ-linolenic acid (4).

Earlier studies showed that pyrrolizidine alkaloids are not co-extracted with the oil. A GC method, capable of measuring ppm levels, and TLC methods, capable of measuring even ppb levels, were used in these studies (Refs. 1, 5, and 6, respectively). However, before using borage oil for pharmaceutical purposes, the pyrrolizidine alkaloid content must first be determined to ensure the absence of these compounds. This requires a less elaborate but more selective and sensitive method than TLC. According to a specification limit, established in 1988 by the German Federal Health Agency, the pyrrolizidine alkaloid content in pharmaceutical products should be equal to or less than 100 ppb, or 0.1 μg/g (7). In 1992, limits were finally established for the daily intake of pyrrolizidine alkaloids. For registered products the total daily intake should be less than 1 μg, and for nonregistered products this limit should be 10-fold lower, i.e., 0.1 μg (8).

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It is also important to examine the capacity of the refining process to remove the pyrrolizidine alkaloids from the crude oil. Hence, if the level for pyrrolizidine alkaloids in the oil is low and the refining process reduces the pyrrolizidine alkaloid content significantly, only negligible levels of the hepatotoxic pyrrolizidine alkaloids would be expected in the final product.

A standard method for extracting pyrrolizidine alkaloids from oil is described in a thesis by Huizing (9), beginning with extraction by sulfuric acid and chloroform. The organic phase is then discarded, and the water phase, containing pyrrolizidine alkaloids in free and oxidized form, is subjected to zinc reduction. Subsequently, the aqueous phase is made alkaline and the pyrrolizidine alkaloids are back-extracted into a fresh portion of chloroform.

Soxhlet extraction is normally used for the extraction of pyrrolizidine alkaloids from solid matrices such as flowers or seeds of the plant *B. officinalis*. However, it has been shown that supercritical fluid extraction (SFE) can be used also. This extraction technique does not consume much solvent and thus reduces the levels of contaminants (10). High-speed countercurrent chromatography also has been applied for the preparative separation of pyrrolizidine alkaloids from plant material (11). Chloroform was used as a mobile phase, and the stationary phase was an aqueous phosphate buffer.

The most commonly applied technique for the determination of pyrrolizidine alkaloids is GC–MS with electron or chemical ionization (EI and CI, respectively). A combination of the positive ion CI (PICI) and negative ion CI (NICI) techniques has been shown to be useful in this context when performed on samples subjected to silyl derivatization. Here, the PICI mass spectra reflect the fragmentation of the retronecine base, and the NICI mass spectra reflect the fragmentation of the necin acid (12). However, difficulties might arise in obtaining a complete derivatization of pyrrolizidine alkaloids that possess two adjacent hydroxy groups, especially the heterocyclic forms. The most effective chromatographic separation of the pyrrolizidine alkaloids containing two hydroxy residues is obtained when they are transformed to alkyl boronate derivatives (13). These derivatives produce ions of high abundance and prominent molecular ions when analyzed by GC–MS using EI (14,15). However, in cases where a single, isolated hydroxy group is present, as in lycopsamine/intermedine, the chromatographic performance might be impaired since excess alkyl boronic acid can interfere with the single hydroxy group. This problem can be solved by derivatization of the hydroxy group (13,15). The alkyl boronic acid

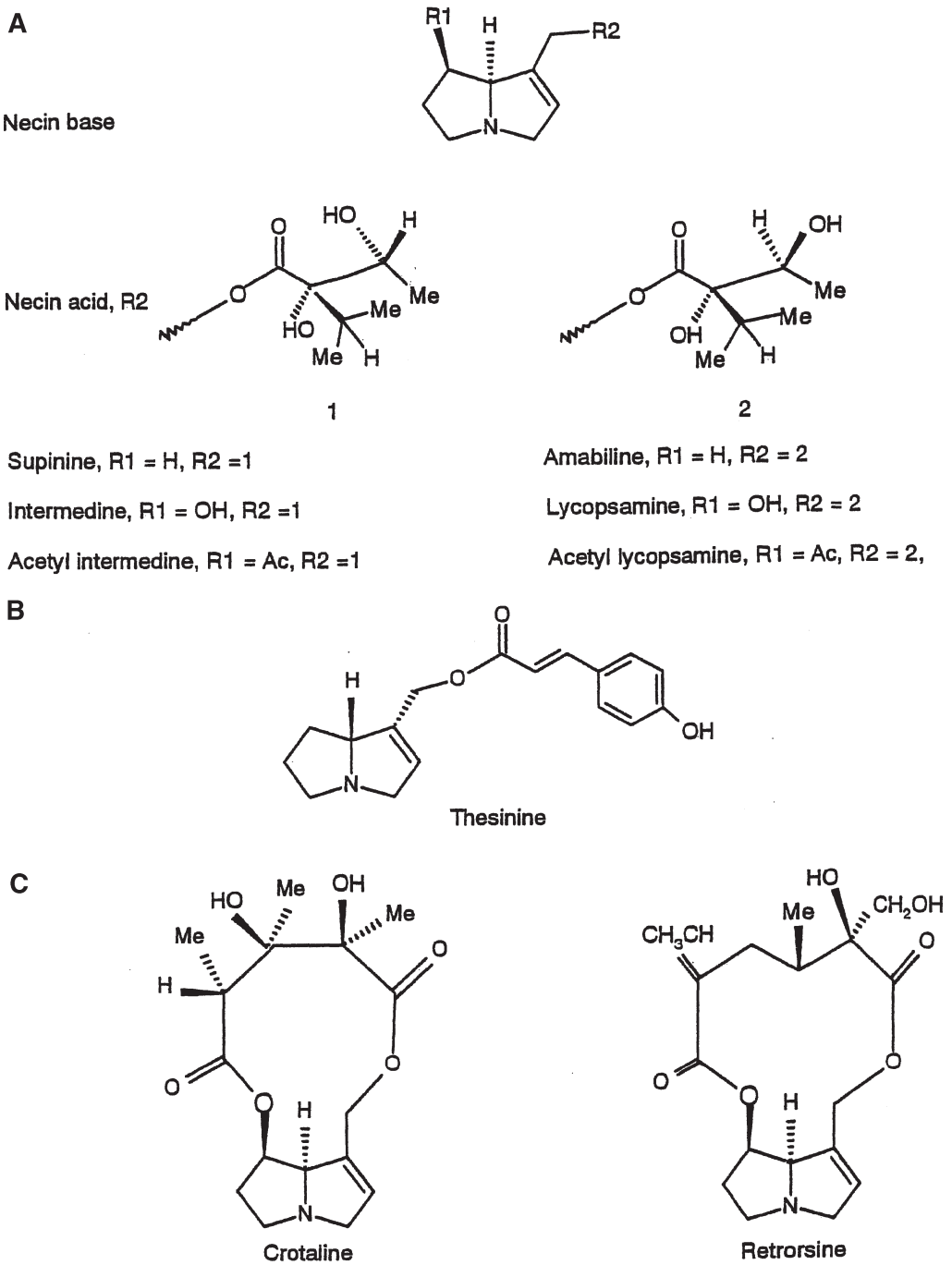

FIG. 1. Chemical structures of (A) pyrrolizidine alkaloids found in the seeds of *Borago officinalis,* (B) thesinine, and (C) crotaline and retrorsine, the internal standards.

derivatization could advantageously be followed by acetylation or silylation. Among the alkyl boronates, the butyl boronate derivatives are considered the most suitable with respect to properties like volatility and stability (15). These derivatives also can be used for quantification by GC using FID.

The aim of this study was to determine the abundance of pyrrolizidine alkaloids in crude borage oil and in oil from different stages of processing. The crude oil was analyzed by GC–MS. This technique was used because of its inherent capability to combine high sensitivity with the possibility of identifying analytes. Furthermore, reduction factors for pyrrolizidine alkaloids in the oil refining process were determined by means of spiking experiments. These samples were analyzed by GC–FID.

EXPERIMENTAL PROCEDURES

Materials. Crude borage oil, processed through solvent extraction with hexane, was obtained from Karlshamns Oljefabriker (Karlshamn, Sweden). Batches of processed and partly

processed borage oil were produced at laboratory scale at Pharmacia AB (Stockholm, Sweden). Flowers, freeze-dried and milled, were obtained from Karlshamns Oljefabriker. The pyrrolizidine alkaloid standards crotaline and retrorsine were bought from Sigma (Stockholm, Sweden), as were the reagents 1-butane boronic acid and 2,2-dimethoxypropane. Extra-pure reagents, such as chloroform of B&J Brand quality from Fluka (Buchs, Switzerland), sulfuric acid 95–97% p.a., and ammonia solution (25% Suprapure) from Merck (Darmstadt, Germany) were required for the extraction. Zinc powder p.a. and pyridine were obtained from Merck. The silylation reagent, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), was purchased from Pierce (Rockford, IL). Finally, for the processing, concentrated phosphoric acid (85%), sodium hydroxide, bleaching earth (Tonsil LFF80; Süd-Chemie, Munich, Germany), and citric acid were required.

Sample preparation for determination of the specification limit. A solution (1 mL) at a concentration of 1 μg crotaline per mL chloroform was added to 10 g of the oil sample, resulting in a crotaline content of 100 ppb. The crotaline was then isolated using liquid–liquid extraction as described below. Sample preparations without addition of crotaline were also made. The extracts were stored in a freezer before GC–MS analysis.

Liquid–liquid extraction. Pyrrolizidine alkaloids were isolated from borage oil by liquid–liquid extraction. Ten grams of the oil sample was diluted in 100 mL of chloroform and the organic phase was extracted with 1 M sulfuric acid, 1 × 50 mL and 2 × 25 mL. Zinc powder (2 g) was then added to the combined water volumes to perform a reduction, and the organic phase was discarded. Subsequently, the aqueous phase was filtered and made alkaline (pH 9.5) by the addition of ammonia solution (NH_3, 25%). The solution was extracted with chloroform, and the aqueous phase was discarded. The chloroform was removed by vacuum evaporation at 40°C. During the evaporation process, 2,2-dimethoxypropane (1 mL) was added to the extract to serve as a water scavenger, and the evaporation was resumed.

The pyrrolozidine alkaloid extract was derivatized by the addition of a solution of 1-butane boronic acid in chloroform. Equal amounts of pyrrolizidine alkaloid extract and reagent were mixed (corresponding to a mole ratio of about 1:3). After evaporation of the chloroform, the extract was silylated with 50 μL BSTFA in 50 μL pyridine (1:1). The mixture was then incubated at 80°C for 40 min. Subsequently, the silylation reagent was evaporated and a suitable volume of chloroform was added. The sample preparations were stored in a freezer before GC–MS analysis.

GC–MS analysis. For the GC–MS analysis, a Hewlett-Packard HP 5890 gas chromatograph coupled to a Fisons VG 30-250T mass spectrometer was used. The falling-needle injector technique was applied to improve the sensitivity of the analysis, enabling injection of larger sample volumes. A combination of two columns, DB-1 and DB-17 (J&W Scientific, Folsom, CA), 0.18 mm i.d. and 0.3 μm df, each 5 m long, was considered suitable because of its ability to separate the pyrrolizidine alkaloids from plasticizers originating from the crude oil.

The pressure of the carrier gas, helium, was set to 8 psi. The injection volume varied between 1 and 10 μL, and the injection temperature was 200°C. The initial oven temperature was held at 200°C for 1 min and then increased by 5°C/min to a temperature of 310°C, at which it was maintained for 5 min. The temperatures of the ion source and interface were 200 and 260°C, respectively, and the ionization energy was 70 eV.

Both total ion current (TIC) and selected ion monitoring (SIM) were used. The fragments selected for SIM, *m/z* 119, 120, 136, and 210, are typical of unsaturated pyrrolizidine alkaloids. This means that if borage pyrrolizidine alkaloids were present they would be quantified too, together with crotaline. The sensitivities of both TIC and SIM were examined by injection of standard solutions in the range of 0.1–10 μg/mL.

To confirm that the GC–MS analysis had the capacity to identify and quantify pyrrolizidine alkaloids typical of *B. officinalis*, pyrrolizidine alkaloids were isolated from flowers of this plant by means of SFE and analyzed by GC–MS.

SFE of flowers of B. officinalis. Pyrrolizidine alkaloids were isolated from flowers of *B. officinalis* by SFE. The supercritical fluid extractor was an SFX 220 system from ISCO, equipped with a 30 cm × 50 μm i.d. restrictor of fused silica. The flowers were freeze-dried and milled. The flower preparation (0.5 g) was placed in a metal cartridge and 80 μg of crotaline dissolved in 0.8 mL of methanol was added, the solvent here acting as a modifier. The cartridge (volume, 10 mL) was sealed and placed in a supercritical fluid extractor. The extraction program started with a static period of 1000 s (16.6 min) followed by a dynamic period of 50 min. The pressure was maintained at 3000 psi, and the extractor volume was 30 mL. The oven temperature and restrictor temperature settings were 55 and 60°C, respectively.

The final extract was collected in a test tube containing 2 mL of methanol. After evaporation of the solvent, the residue was prepared according to the procedure for oil samples described previously. The sample preparations were stored in a freezer before GC–MS analysis.

Solubility of crotaline in borage oil. Prior to the spiking experiments, the solubility of pyrrolizidine alkaloids in borage oil was determined. This was done to certify the homogeneity of the added crotaline solution. Crotaline, dissolved in chloroform, was added to the crude borage oil at different concentrations in the range 10 to 1000 ppm, and the solvent was evaporated under vacuum at 50°C. The mixture was then stirred for half an hour under nitrogen and, finally, filtered through a 0.45-μm Teflon filter. The filtered oil samples were prepared as described previously and analyzed by GC.

Alkali refining. A solution of crotaline in chloroform (50 mL; 1 mg/mL) was added to approximately 0.25 kg of crude oil. The solvent was evaporated under vacuum at 50°C, and the mixture was transferred to a refining vessel. The evaporating flask was rinsed with crude borage oil, which was then added to the refining vessel. Additional oil was added to the refining vessel to a weight of approximately 1.0 kg. This oil

mixture was mixed for 30 min after the addition of crotaline. Samples from the crude oil and from the spiked oil were collected for subsequent analysis.

The alkali refining was preceded by conditioning with phosphoric acid to ensure that all the phosphatides, as well as the magnesium and calcium salts of phosphatidic acid, had been removed with the soapstock. To the spiked oil, concentrated phosphoric acid (1.0 mL) was added, and the mixture was stirred for 5 min under a nitrogen atmosphere. The temperature was held at 80–85°C during the conditioning. To neutralize the oil, 4 M sodium hydroxide (47 mL) was added to the hot mixture, which was then stirred for 10 min in a nitrogen atmosphere while the temperature was maintained at 80-85°C. When the reaction was complete, the oil mixture was cooled in an ice-water bath to ambient temperature under a nitrogen atmosphere while being stirred. The oil was then transferred to centrifuge vessels and centrifuged (5 min), after which it was carefully decanted from the soapstock and weighed. Subsequently, the oil was washed by addition of water at a temperature of 80–90°C with gentle stirring. The oil was allowed to separate from the water, which was discarded. In total, the washing procedure was performed five times using a water volume equal to that of the oil. The separation times for the first four washings were 5 min and for the last one, 30 min. To remove residual water, the oil was vacuum-dried at a temperature of 80°C for 35 min. Finally, the oil was cooled to a temperature below 30°C, and samples were collected for determination of the pyrrolizidine content after alkali refining.

Bleaching. Crude oil (1.0 kg) was subjected to the alkali refining process as described above. A solution containing crotaline in chloroform (50 mL; 1 mg/mL) was added to approximately 250 g of refined oil. The solvent was evaporated under vacuum at 50°C, and the mixture was transferred back to the refining vessel containing the alkali-refined oil. The evaporation flask was rinsed with 250 g of refined oil, previously removed, and this was also returned to the refining vessel. The oil mixture was continuously stirred for 30 min after the addition of crotaline. Samples from the alkali-refined oil and from the spiked oil were collected for subsequent analysis.

Before bleaching, the oil, which amounted to approximately 1 kg, was treated with citric acid by the addition of a solution of citric acid in ethanol (0.8 mL; 25%). The reaction vessel was placed under vacuum, and the mixture was allowed to react for 30 min. The temperature was raised to a maximum of 80–85°C during the reaction. The vacuum was then released by admitting nitrogen and bleaching earth (8.5 g). The vacuum was restored, and the mixture was allowed to react for 30 min while the maximal temperature was maintained at 80–85°C. After releasing the vacuum by introducing nitrogen, the oil mixture was cooled to ambient temperature in a water bath. This was done with stirring in a nitrogen atmosphere. Finally, the mixture was filtered under pressure (cellulose filter with active carbon; Lenzing Technik, Lenzing, Austria). Samples for determination of the pyrrolizidine content after bleaching were collected.

Deodorization. Approximately 1.0 kg of crude oil was processed by alkali refining and bleaching, as described previously. The oil was stored in a refrigerator before deodorization. A crotaline solution (50 mL; 1 mg/mL) was added to the processed oil (250 g) and the solvent evaporated under vacuum at 50°C, whereupon the mixture was transferred to a mixing chamber. The evaporation flask was rinsed with an aliquot of the processed oil and added to the mixing chamber, together with additional oil up to an approximate amount of 1.0 kg. The oil mixture was stirred continuously for 30 min after the addition of crotaline. Samples from the processed oil and from the spiked oil were collected for subsequent analysis.

The oil mixture was transferred to a deodorization vessel where it was preheated to about 50°C and degassed. The reaction vessel was then placed under vacuum at a maximum pressure of 5 mbar. The temperature was raised to a maximum of 220 ± 4°C, and stripping steam in an amount of 9%, based on the weight of the oil, was allowed to pass through the oil for 2 h. After deodorization was finished, the oil was cooled to a temperature of 50°C and the vacuum was released by admitting nitrogen. Samples for determination of the pyrrolizidine content after deodorization were collected.

A flow diagram for the processing and sampling performed in the spiking experiments is given in Table 1. The amounts of starting material originated from three different batches.

Preparation of samples collected from the spiking experiments. Retrorsine (1 mL; 100 μg/mL) was added to the oil sample (10 g) as an internal standard. The spiked pyrrolizidine alkaloid, crotaline, was then isolated using liquid–liquid extraction as described previously in connection with the analysis of crude oil, and the extracts were stored in a freezer before GC–FID analysis.

GC–FID analysis. For GC–FID analysis, an HP 5880 gas chromatograph equipped with an on-column injector and an FID was used. A CPSil 5CB column from Chrompack (Middelburg, The Netherlands), 30 m × 0.32 mm, 0.25 μm df, connected to a retention gap of 2.5 m × 0.53 mm, was installed in the GC. The sample (1 μL) was injected into the GC. The temperature was held at 70°C in both injector and oven during the injection. The oven temperature was then programmed to rise 20°C/min to 175°C, followed by 5°C/min up to 300°C, which was maintained for 10 min. The injector temperature was also programmed to increase from 70 to 300°C at a rate of 80°C/min after a delay of 0.05 min. A constant gas flow of 1.5 mL/min was maintained throughout the analysis. The detector temperature was 330°C.

RESULTS AND DISCUSSION

GC–MS of crude and refined borage oil. Pyrrolizidine alkaloids were determined in two batches of crude borage oil, to which 100 ppb of crotaline had been added. Triplicate analyses were performed. It was concluded that no pyrrolizidine alkaloids appeared at concentrations above the 100 ppb limit. In the TIC and extracted ion chromatograms obtained, as well

TABLE 1
Flow Diagram of the Processing and Sampling Steps

Processing step	Alkali refining	Bleaching	Deodorization
Amount of starting material	1 kg	1 kg	1 kg
Procedure	1. Sampling 2. Addition of crotaline 3. Sampling 4. Alkali refining 5. Sampling	1. Alkali refining 2. Sampling 3. Addition of crotaline 4. Sampling 5. Bleaching 6. Sampling	1. Alkali refining 2. Bleaching 3. Sampling 4. Addition of crotaline 5. Sampling 6. Deodorization 7. Sampling

as in the selected ion chromatograms, the only pyrrolizidine alkaloids found were crotaline itself and one or two small peaks derived from crotaline (marked C1 and C2 in the chromatograms; Figs. 2B, 3A, and 3B). The peaks probably corresponded to mono- or disilylated crotaline that had not bound to the butane boronic molecule. No pyrrolizidine alkaloids were found in the chromatograms from the analysis of borage oil without added crotaline. In the TIC chromatograms resulting from analysis of the crude oils, some plasticizers were found, e.g. dibutyl phthalate (DBP), dioctyl phthalate (DOP), and squalene (Fig. 2A). Therefore, extraction ion chromatograms of the ions typical of pyrrolizidine alkaloids were also collected in addition to the TIC chromatograms to obtain a better understanding of the nature of the small peaks in the chromatogram (Fig. 2B). Unfortunately, squalene also appears in the extracted ion chromatogram as a large peak at a scan time of about 13 min, owing to the presence of peaks at *m/z* 119 and 136 in its spectrum. A SIM chromatogram obtained from analysis of preparations of the same batch is shown in Figure 3A. The crotaline peak and the peaks C1 and C2 are clearly shown in the chromatogram. In addition, two batches of refined oil, corresponding to the batches of crude oil analyzed previously, were analyzed, also in triplicate, by SIM, and one of these chromatograms is shown in Figure 3B. The refined oil originates from the crude oil represented by Figures 2A and 2B. The chromatogram did not contain any signs of pyrrolizidine alkaloids.

TIC monitoring was used for the identification of pyrrolizidine alkaloids, and spectra were collected for each peak. SIM was used to determine the specification limit of the respective pyrrolizidine alkaloids. The sensitivities of TIC and SIM monitoring were determined to be 100 and 10 ppb, respectively.

An important issue in the analytical performance was to keep the pyrrolizidine alkaloid window of the chromatogram

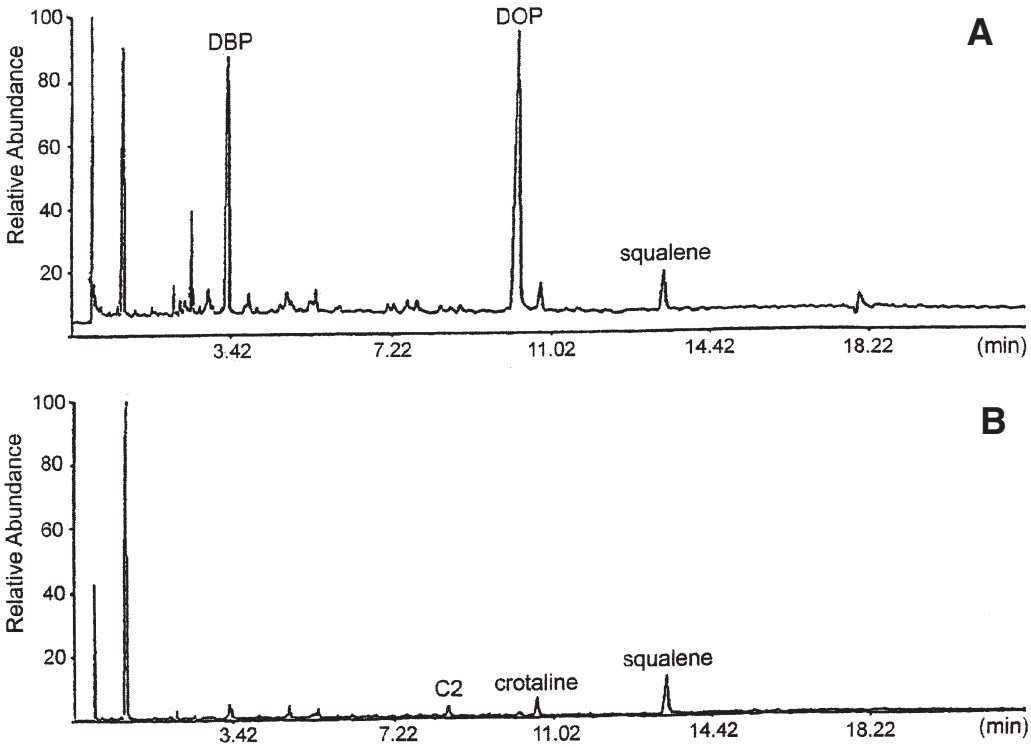

FIG. 2. (A) Total ion current chromatogram and (B) extracted ion chromatogram obtained from the determination of pyrrolizidine alkaloids in crude borage oil. DBP, dibutyl phthalate; DOP, dioctyl phthalate; C2, crotaline derivative.

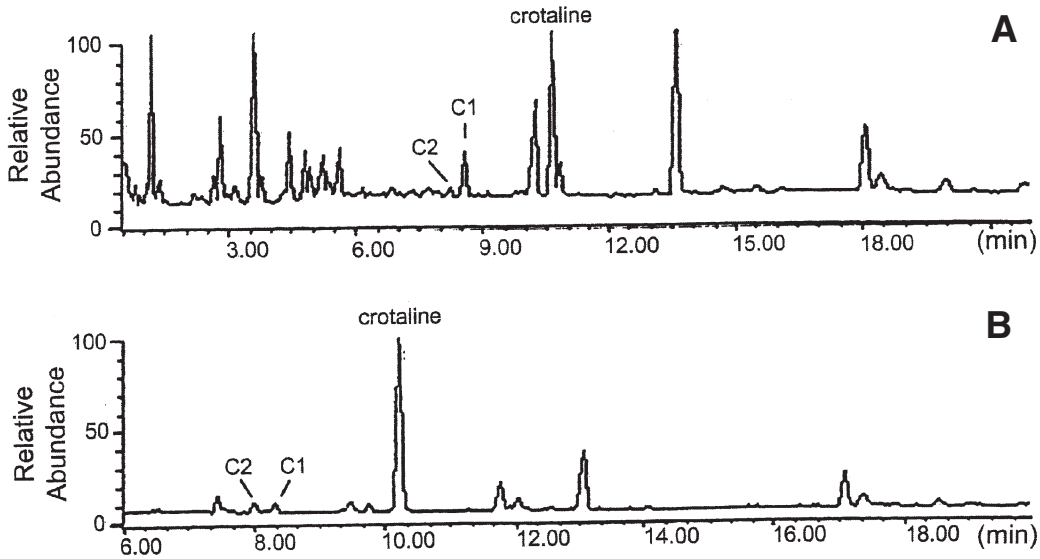

FIG. 3. Selected ion monitoring chromatograms obtained from the determination of pyrrolizidine alkaloids in (A) crude borage oil and (B) refined borage oil. C1, C2, crotaline derivatives.

free from analytical artifacts, the main potentially interfering substances being squalene and plasticizers. However, a slight improvement was achieved by using a combination of a nonpolar and a polar column. Sample cleanup by means of extraction through a C18 solid-phase extraction column had previously been tested and shown to be unsuccessful.

SFE and GC–MS of flowers from B. officinalis. To confirm the results, efforts were made to produce pyrrolizidine alkaloid reference substances. Flowers from *B. officinalis* were extracted by means of SFE to obtain pyrrolizidine alkaloids, but they were not obtained in sufficient amounts for use as standard substances, although a small amount of lycopsamine/intermedine was found (Fig. 4A). The fact that it was possible to isolate and detect a pyrrolizidine alkaloid typical of *B. officinalis* confirms that both the extraction method and GC–MS analysis with the selected ions used were adequate for the purpose. In the spectrum of lycopsamine/intermedine, fragments typical of the necin base were found at *m/z* 94 (68), 119 (2), 120 (33), 136 (7), and 210 (100) (Fig. 4B). The signal from the molecular ion, M^+, at *m/z* 437 was small but distinct, indicating an abundance of 5%. A fragment typical of the necin acid residue was also found at *m/z* 227 (7). The presence of the silyl group in the molecule might affect the fragmentation pattern in favor of the reagent and not the mother molecule.

GC of borage oil from different refining steps. The analytical results of the spiking experiments are shown in Table 2. The crotaline content in borage oil was reduced by a factor of more than 100 during bleaching and about half as much during deodorization. A smaller decrease in the crotaline content was achieved during alkali refining, a step designed for removal of phosphatides and FFA. Only a small decrease was expected in this initiating step since it is focused mainly on removal of acid components from the oil. Some adsorption to the soapstock might, however, be expected. The largest reduction was accomplished by the bleaching step, the main purpose of which is to remove pigment components such as chlorophyll and carotenoids. It has been shown that these pigments are present in the oil in colloidal form and are precipitated by means of the bleaching earth (17). Other unwanted components, comprising metals, sulfur compounds, and peroxides are also removed by bleaching. Deodorization also effectively reduces the pyrrolizidine alkaloid content. Components having partial pressures equal to or exceeding those of the common FA, e.g., flavoring compounds, are removed during deodorization. In addition to physical properties of the components such as the partial pressures, the degree of removal also depends on the temperature and volume of steam passed through the oil. It is well known that other contaminants such as pesticides are removed during deodorization. For the whole process, a reduction factor of 30,000 was obtained, indicating that pyrrolizidine alkaloids, if present in crude oil, would be substantially reduced by the refining process.

Before the spiking experiments, the solubility of pyrrolizidine alkaloids in borage oil was examined. The results of the solubility tests showed that it is possible to dissolve up to 1,000 ppm crotaline in crude borage oil. For the spiking experiments, a level of 50 ppm was chosen since only extremely low levels of pyrrolizidine alkaloids would be expected in crude borage oil, and the intention was to perform the experiments under realistic conditions. However, it should be high enough to be determined with good reproducibility after an expected reduction in the processing step. In Table 2 the results from the spiking experiments show that higher values than expected, about 70 ppm, are obtained after spiking both before bleaching and before deodorization. This may be explained by oil losses during the preceding part of the processing.

The validation results of the analytical method, involving isolation by liquid–liquid extraction and subsequent GC–FID

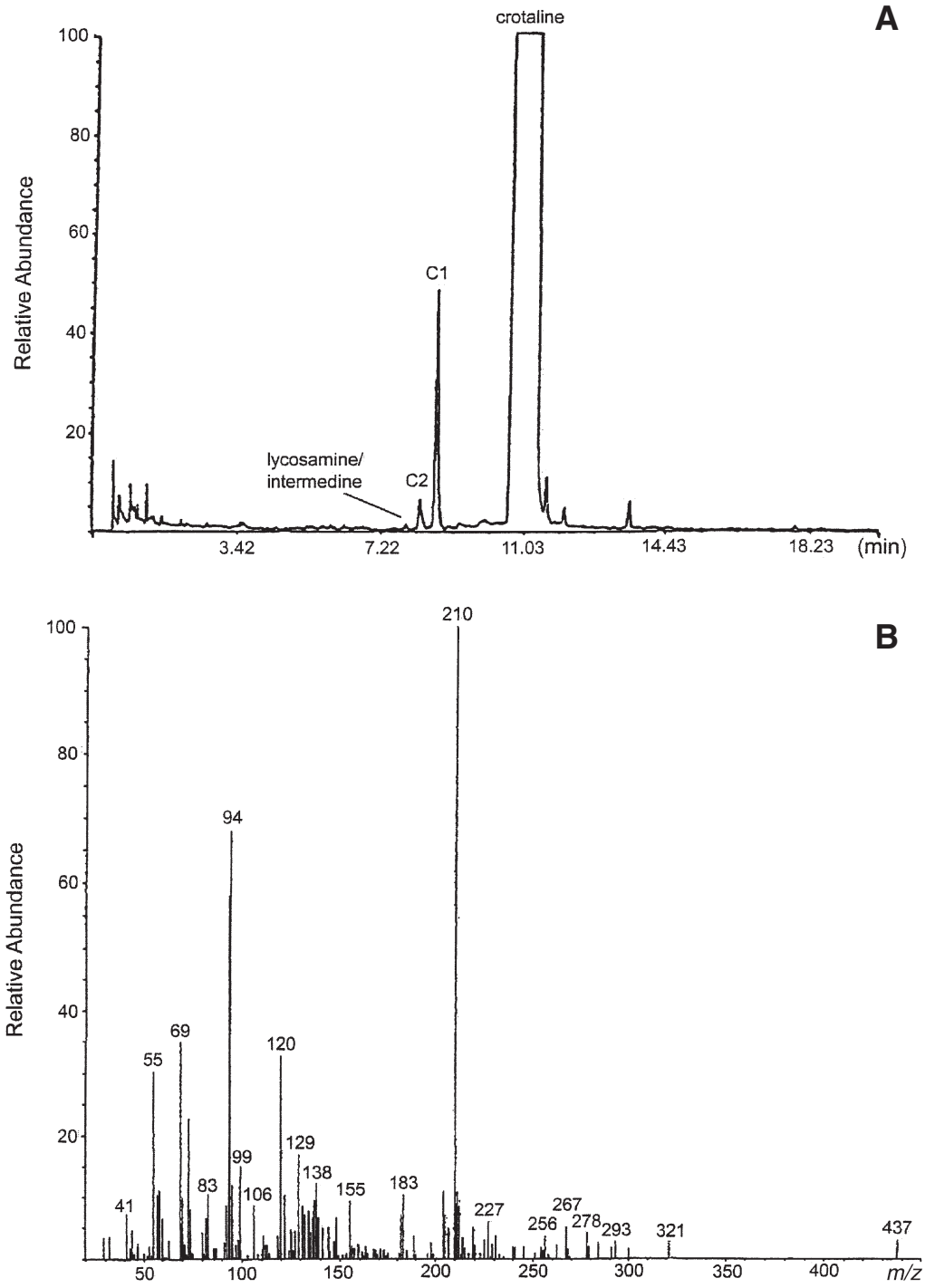

FIG. 4. (A) Extracted ion chromatogram and (B) mass spectrum of lycopsamine/intermedine obtained from the determination of pyrrolizidine alkaloids in flowers from *Borago officinalis*.

analysis, showed that the method was reliable. In an investigation of linearity in the concentration range 1 µg/mL to 1 mg/mL, the derived correlation coefficient was 1.000 and the intercept –0.063. A correlation coefficient was also calculated for the narrow range 1–50 µg/mL, to ensure the reliability at lower concentrations, and was found to be 0.999. The limit of detection of the method of analysis was 0.2 µg/mL (3S/N), corresponding to a crotaline content of 20 ppb in borage oil. The reproducibility was investigated using five different samples of crude oil with added crotaline, 10 ppm. A relative SD of 10% was obtained, and the recovery was 99%. Note that crude oil represents the most complex matrix of the ones investigated.

In summary, it has been shown by GC–MS analysis that no pyrrolizidine alkaloids are present in crude borage oil at levels above the 100 ppb limit. These results are in accordance with

TABLE 2
Effect of the Individual Processing Steps on the Content of Pyrrolizidine Alkaloids in Borage Oil—Determination of Reduction Factors

	Crotaline content (ppm)		
	Determination 1	Determination 2	Average
Alkali refining			
Crude borage oil	<0.02	<0.02	<0.02
Crude borage oil after spiking	55	53	54
Alkali refined borage oil	14	14	14
Reduction factor			4
Bleaching	Determination 1	Determination 2	Average
Alkali-refined oil	<0.02	<0.02	<0.02
Alkali-refined oil after spiking	76	65	71
Bleached oil	0.6	0.6	0.6
Reduction factor			120
Deodorization	Determination 1	Determination 2	Average
Bleached oil	<0.02	<0.02	<0.02
Bleached oil after spiking	77	72	74
Deodorized oil	1.2	1.1	1.2
Reduction factor			60

previous studies (5,6). Furthermore, it has also been shown by spiking experiments that the pyrrolizidine alkaloid content in crude borage oil, if any, is reduced by a factor of about 30,000 by the refining process. This implies that a pyrrolizidine content at a ppb level would be reduced to a ppt level.

The analytical method has been shown to be suitable for the purpose. We have been able to detect pyrrolizidine alkaloids at a level of 20 ppb. The technique also can be used on a routine basis for regular control of batches of oil.

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