Determination of Bromethalin in Commercial Rodenticides Found in Consumer Product Samples by HPLC–UV–vis Spectrophotometry and HPLC–Negative-Ion APCI–MS

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Abstract

A small amount of green particulate material is encountered in a consumer complaint sample. The green particulates in the sample are identified as a bromethalin-containing rodenticide using high-performance liquid chromatographic (HPLC)–UV–vis spectrophotometric and HPLC–negative-ion atmospheric pressure chemical ionization (APCI)–mass spectrometric (MS) approaches, which are commonly used for the detection and confirmation of bromethalin in grain-based rodenticides. The selective and sensitive nature of the MS detector makes it possible to determine bromethalin without extensive sample cleanup and preconcentration. The estimated detection limit with the UV–vis detector is 500 pg of bromethalin injected into the column. The extensive fragmentation of the bromethalin molecule under APCI conditions provides sufficient structural information for positive identification.

Introduction

Commercial rodenticides have appeared in various foods, pharmaceuticals, and other products regulated by the Food and Drug Administration through accidental or intentional means. The Forensic Chemistry Center is frequently asked to analyze samples of pharmaceuticals and foods that have accidentally or intentionally been contaminated with small amounts of green, blue, or tan particles. Based on a characteristic appearance and the color of the contaminant, the initial suspicion is that the particles are grain-based commercial rodenticides. In such tampering cases, it is absolutely essential that adulterants be unambiguously identified when present or that adulteration be ruled out with certainty when a suspected tampering has in fact not occurred. Because a false negative or false positive could have costly consequences, a mass spectrometric (MS) confirmation of identity is generally required.

The commonly used active ingredients of such rodenticides are the anticoagulant classes 4-hydroxycoumarins and indanediones. Methods have been developed in this laboratory for the determination and identification of indanediones and 4-hydroxycoumarins (1,2). This laboratory recently received a consumer complaint sample contaminated with a small amount of green particulate material. Methods developed for the identification of indanediones and 4-hydroxycoumarins indicated that the sample was negative for these classes of compounds. The green particulate material in the sample was subsequently identified by liquid chromatography (LC)–MS as a bromethalin-containing rodenticide, which had not been encountered previously in this laboratory.

Bromethalin (*N*-methyl-2,4-dinitro-*N*-(2,4,6-tribromophenyl)-6-(trifluoromethyl)-benzamine) is a Class 6 poison (extremely toxic) with an LD_{50} of 2 mg/kg for rats. Its toxic effects are associated with its central-nervous-system-suppressing properties (unlike indanediones and coumarins, which act as anticoagulants). It is the active ingredient of some grain-based commercial rodenticides (bait) marketed under various trade names around the world. Because of the acute toxicity of these rodenticides and their ease of availability, there have been many reports of misuse and accidental ingestion of commercial rodenticides by humans and other vertebrates (4,5).

Bromethalin is not amenable to analysis by gas chromatography because of thermal degradation. There have been a number of high-performance liquid chromatographic (HPLC) methods reported in literature for the determination of anticoagulants in commercial rodenticides (6–8) in blood and tissue samples (6,8–10). The methods of analysis for bait formulations typically involve large sample sizes, extensive extraction, cleanup, and concentration. In cases submitted to our laboratory, the amount of sample available for analysis is often very limited. We have identified rodenticides in samples as low as 500 mg.

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No method was found in the literature for the detection and confirmation of bromethalin in grain-based commercial rodenticides. This work describes a simple method for the detection and quantitation of bromethalin in grain-based commercial rodenticides. The sample was sonicated in acetonitrile for 10 min and analyzed by using HPLC with UV–vis spectrophotometric detection. MS confirmation was obtained by analyzing a second aliquot by HPLC–atmospheric pressure chemical ionization (APCI)–MS.

Experimental

Apparatus

The HPLC–UV–vis spectrophotometry system consisted of a Model 1090 LC equipped with a photodiode array detector, an autosampler, and a Rheodyne injector (all obtained from Hewlett Packard, Palo Alto, CA). The sonicator was a Model 08849-00 from Cole-Parmer (Niles, IL).

The HPLC–APCI–MS system consisted of a Model 1100 LC equipped with an autosampler and a Rheodyne injector from Hewlett Packard and a Finnigan (San Jose, CA) LCQ equipped with an APCI source.

Reagents

HPLC-grade acetonitrile was obtained from Fisher Scientific (Pittsburgh, PA). HPLC-grade water was prepared using a Milli-Q water purification system (Millipore Corporation, Bedford, MA) and a Culligan Aqua-Cleer reverse osmosis system (Culligan Corporation, Northbrook, IL). A bromethalin standard (99.6% pure) was provided by courtesy of Purina Mills (St. Louis, MO).

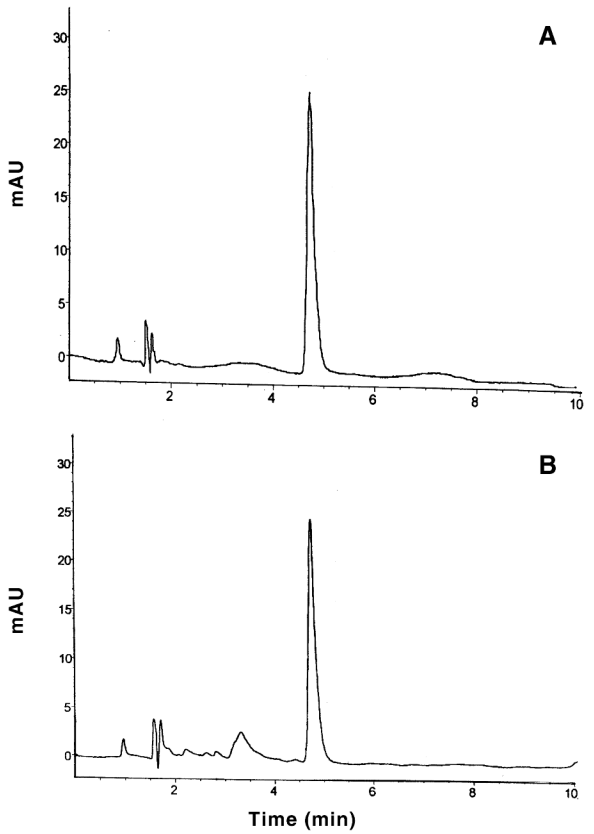

Figure 1. (A) HPLC traces at 215 nm of a 10-µL injection containing 10 ng/µL of a bromethalin standard. (B) HPLC traces of a 10-µL injection of the extract of B1.

Sample preparation

Pellets of commercial rodenticides from three different manufacturers (referred to in this paper as brands B1, B2, and B3) were ground using a mortar and pestle. The labels on all three brands declared that they contained 100 µg/g bromethalin. Approximately 100 mg of sample was mixed with 2 mL of acetonitrile in a vial (4-mL vials were obtained from National Scientific Co., Lawrenceville, GA) and sonicated for 10 min. The mixture was then shaken for 5 min and centrifuged for 3 min in a bench-top centrifuge. An aliquot of the supernatant was filtered through a 0.2-µm nylon or polytetrafluoroethylene filter.

Preparation of standard

A 1000-µg/mL stock standard in methanol was prepared. A working standard was also prepared to match the expected concentration of analyte in the samples by making dilutions in the HPLC mobile phase.

LC conditions

The column was a 2.1-mm × 25-cm (5-µm particle size) Supelcosil LC-18 from (Supelco Inc., Bellefonte, PA). The mobile phase consisted of 85% acetonitrile and 15% water at a flow rate of 0.35 mL/min. Injections of 5 µL each were made in all cases. UV–vis spectra from 210 nm to 450 nm were stored, and UV signals were recorded at 350 and 215 nm. Quantitation was done

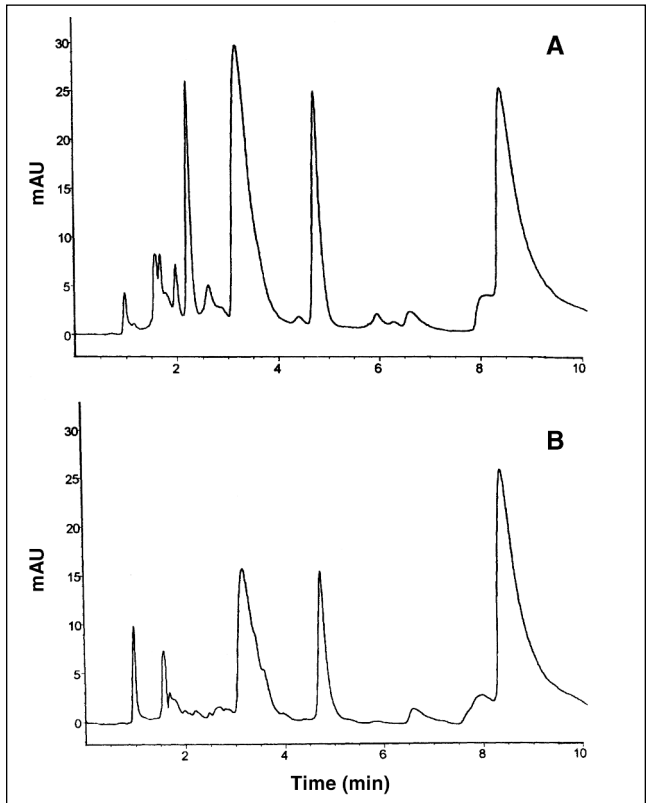

Figure 2. (A) HPLC traces at 215 nm of a 25-µL injection of the extract of B2. (B) HPLC traces of a 25-µL injection of the extract of B3.

using a one-point calibration, and the concentration of this standard (6 ng/mL) was chosen to match the expected concentration of bromothalin in the extracts. The response at 350 nm was used for quantitation.

MS conditions

The capillary voltage was set at –10 V. The vaporizer temperature and capillary temperature were set at 450°C and 150°C, respectively. The normalized collisionally induced dissociation (CID) energy was 30% and the ionization mode negative.

Procedure

Filtered samples were analyzed by HPLC with UV–vis detection for quantitation and tentative identification. A second aliquot was analyzed by HPLC–negative-ion APCI–MS for confirmation and identification.

Results and Discussion

HPLC–UV–vis results

Figure 1A shows a UV–vis chromatogram of a bromethalin standard at 215 nm. Bromethalin has a strong broad absorption band extending from 300 to 415 nm in the mobile phase, which provides useful quantitative information. Figures 1B, 2A, and 2B show the corresponding chromatograms for the three brands of commercial rodenticides declared to contain bromethalin. It should be noted that from the UV–vis chromatogram at 215 nm, one can differentiate among the three brands. Such chemical profile information is useful in tampering investigations because it can be used to identify the brand used in the tampering. There was no significant difference between the chromatogram for the standard and the samples at 350 nm (the wavelength used for quantitation). The chromatogram at 350 nm provided a relatively simple chromatogram despite the minimal sample preparation.

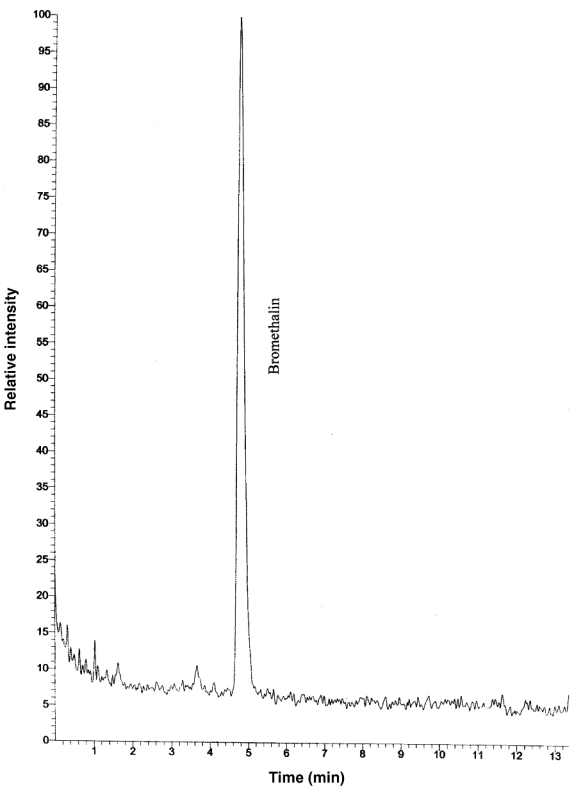

Figure 3. TIC of a 10-µL injection containing 10 ng/µL of a bromethalin standard.

Five duplicate sample preparations from each brand were analyzed to determine method validity. The levels of active ingredient found in B1 and B2 were 80% and 90% of the declared values, respectively. Also, the relative standard deviations (RSDs) were 3.0% for the two brands. The declared bromethalin concentration in each brand was 100 µg/g. B3 consistently yielded bromethalin levels of 50% or less of the declared value with an RSD of 5%. Re-extracting the sample with the extraction solvent showed trace levels of bromethalin, thus ruling out a significant partitioning of bromethalin between the extraction solvent and the sample matrix. Using two other extraction solvents (isopropyl alcohol or

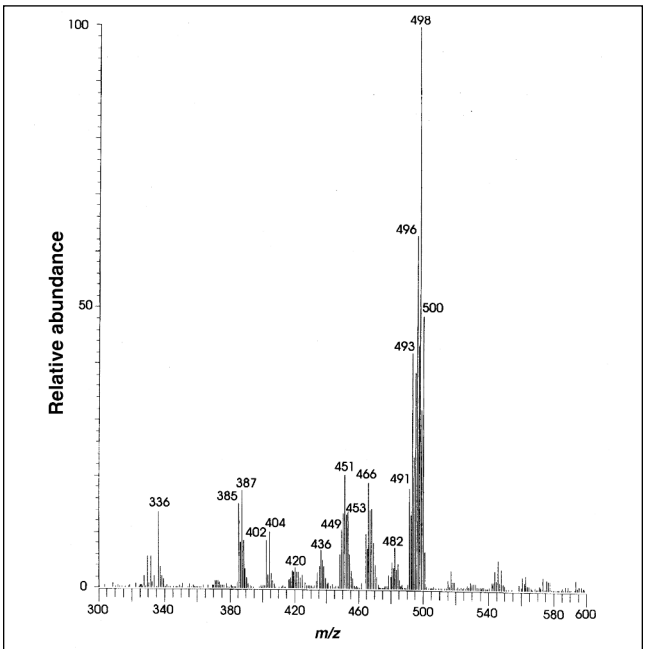

Figure 4. Mass spectrum obtained for a bromethalin standard.

Table I. Summary of Ions Observed in the Mass Spectrum of Bromothalin

m/z	Likely origin
496, 498, 500	$[M\text{-}Br]^-$
495, 497, 499	$[M\text{-}HBr]^-$
465, 467, 469	$[M\text{-}HBr\text{-}NO]^-$
466, 468, 470	$[M\text{-}Br\text{-}NO]^-$
449, 451, 453	$[M\text{-}HBr\text{-}NO_2]^-$
450, 452, 454	$[M\text{-}Br\text{-}NO_2]^-$
433, 435, 437	$[M\text{-}HBr\text{-}NO_2\text{-}O]^-$
434, 436, 438	$[M\text{-}Br\text{-}NO_2\text{-}O]^-$
416–419	$[M\text{-}2Br]^-$, $[M\text{-}HBr\text{-}Br]^-$
419–421	$[M\text{-}HBr\text{-}NO_2\text{-}NO]^-$
420–424	$[M\text{-}Br\text{-}NO_2\text{-}NO]^-$
385, 387	$[M\text{-}2Br\text{-}NO]^-$
386, 388	$[M\text{-}HBr\text{-}Br\text{-}NO]^-$

acidified methanol, which are used in our laboratory for the extraction of anticoagulant rodenticides) also yielded 50% of the declared value for B3 when there was an extraction of better than 90% of the declared value for the other two brands. When pellets from the same packet of B3 were analyzed after two years of storage at room temperature in a Nalgene bottle, less than 10% of the declared values were found. Spike recoveries in B3 ranged from 92–111%. Freshly ground pellets from a second packet with the same code obtained from the same manufacturer at the same time showed bromethalin levels of 82% of the declared value. This could be a result of the possible decomposition of bromethalin once the packets were opened or a bad batch.

The estimated detection limit injected onto the column was 500 pg of bromethalin. This estimate was based on the amount of bromethalin that would produce a signal 2.5 times the peak-to-peak noise in the baseline. This translates to an extraction of 0.5 mg of the pelletized rodenticide using 1 mL of the extraction solvent and an injection of 20 µL of the sample. The estimated quantitation limit was 2 ng on the column or 4 times the detection limit.

HPLC–negative-ion APCI–MS results

The chromatographic conditions for the MS experiment were identical to those used for UV–vis spectrophotometry. Figure 3 shows the total ion chromatogram (TIC) obtained for the bromethalin standard. The full-scan mass spectrum of the standard showed extensive fragmentation and only insignificant abundances of the molecular or $[M-H]^-$ ion, as shown in Figure 4. Only the most prominent and significant ions in the mass spectrum will be discussed. The low intensity of the molecular ion and

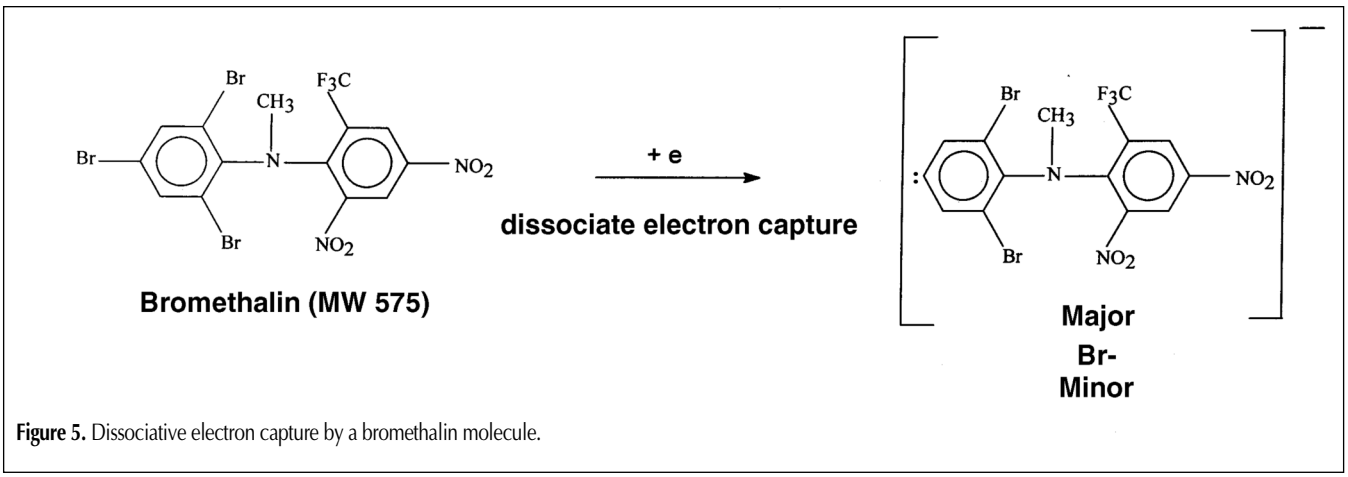

Figure 5. Dissociative electron capture by a bromethalin molecule.

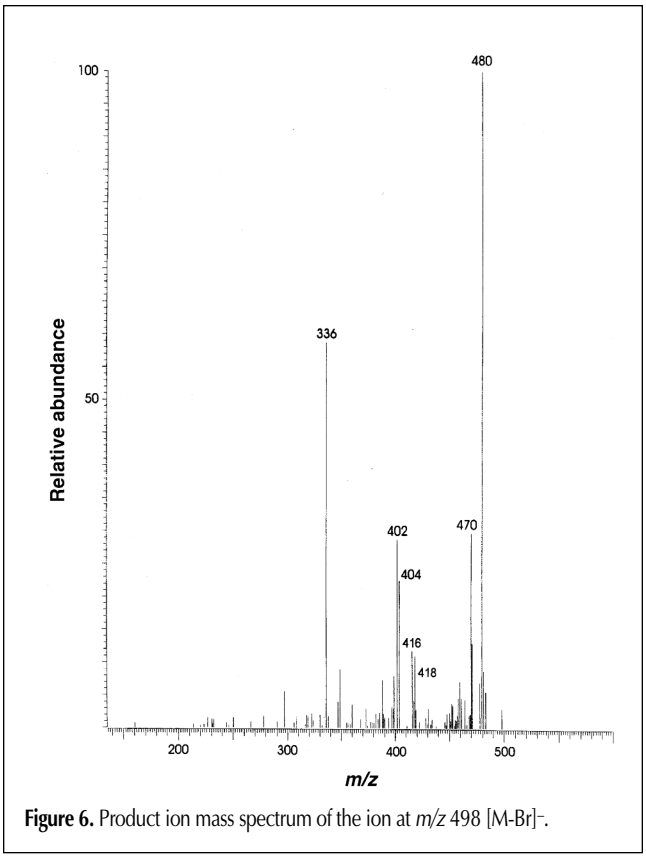

Figure 6. Product ion mass spectrum of the ion at *m/z* 498 $[M-Br]^-$.

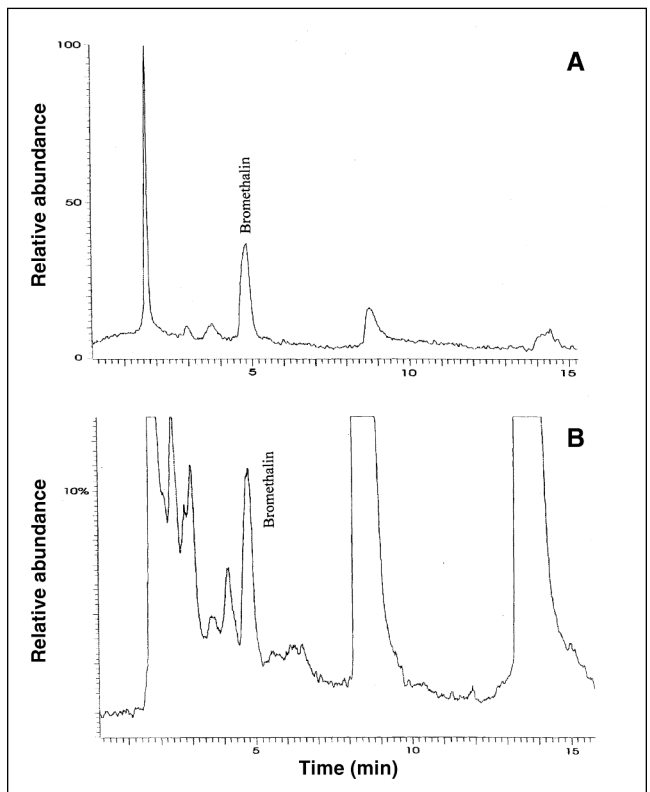

Figure 7. TIC of a 15-µL injection of a rat poison extract: (A) B1 and (B) B2.

the presence of Br^- at *m/z* 79 and 81 (not shown in Figure 4) was consistent with literature reports on the negative-ion methane chemical ionization (CI) mass spectra of triple brominated phenyls (11). The initial ionization step is believed to be a dissociative electron capture (shown in Figure 5).

Table I lists the most significant sets of ions in the mass spectrum of bromethalin and the likely origins of these ions. The most abundant set of ions was at *m/z* 496, 498, and 500. The ion cluster appearance indicates the presence of two bromine atoms in the fragment. This set of ions resulted from the initial dissociative electron capture step. The occurrence of $[M-HX]^-$ next to the $[M-X]^-$ in the spectra of halogenated aromatics is well-documented (12). Losses of NO, NO_2, and O are consistent with literature reports of negative-ion CI mass spectra of nitrophenols (13,14). The complex cluster between *m/z* 416 and 424 is a result of a combination of several steps, as speculated in Table I.

Figure 6 shows the product ion mass spectrum of the ion at *m/z* 498, which indicates two equally abundant ions at *m/z* 402 and *m/z* 404 corresponding with $[M-Br-CH_3Br]^-$. The ion at *m/z* 480 was possibly because of a loss of H_2O. The set of ions at *m/z* 416 and 418 were likely because of $[M-Br-HBr]^-$, and the ion at *m/z* 336 corresponds with $[M-Br-2HBr]^-$. The ion at *m/z* 470 (corresponding with an unexpected loss of 28 from $[M-Br]^-$) was possibly from $[M-Br-NO_2+H_2O]^-$. The relatively long activation times used in the ion trap during the CID step in the presence of back ground water made this possible. The product ion mass spectrum of the ion at *m/z* 500 (all ^{81}Br) showed ions with significant abundances at *m/z* 482, 472, 418, 404, and 336. This shows that the sets of ions at *m/z* 402 and 404 and at *m/z* 416 and 418 involved the loss of two Br atoms, and the ion at *m/z* 336 involved a loss of all three Br atoms from the molecule.

Figure 7 shows the TICs for B1 and B2. The TIC for B3 was similar to that of B2, yet distinguishable from B3. It should be noted that the TICs for the three brands were significantly different. Such information coupled with the UV–vis data may assist in establishing brand identity, which would be useful in tampering cases.

Conclusion

The method described provides a simple and sensitive procedure for the determination of bromethalin in commercial rodenticides found in consumer product samples. The extensive fragmentation of the bromethalin molecule under APCI conditions provides sufficient structural information for positive identification; such information is essential in product tampering and forensic cases. This method provides sufficient selectivity and sensitivity for the confirmation of bromethalin in bait without extensive sample cleanup and preconcentration. From the UV–vis chromatogram at 215 nm and TIC, one can clearly differentiate between different brands—a feature highly desirable in forensic and tampering cases.

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