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Detection of Trace Drugs of Abuse in Baby Formula using Solid Phase Extraction Direct Analysis in Real Time Mass Spectrometry (SPE-DART-MS)

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Keywords:	SPE-DART-MS, Drug Analysis, DART-MS, Baby Formula, Fentanyl, Drug Detection

Abstract

Forensic chemists are being faced with increasingly complex samples to analyze. One of these samples is baby formula that potentially contains illicit drugs. This study investigates the use of solid phase extraction (SPE) coupled with direct analysis in real time mass spectrometry (DART-MS) to efficiently extract and detect five drugs (cocaine, dextromethorphan, fentanyl, heroin, and lorazepam) from baby formula. First, instrumental parameters including DART gas stream temperature and linear speed of the rail sample holder were optimized, and then representative responses and sensitivities for the five drugs were established. Sensitivities were found to be in the single to hundreds ng/mL range, which is well below estimated lethal doses. The presence of baby formula was found to increase analyte signal (relative to pure methanol solutions). Comparison of the SPE-DART-MS method to a traditional DART-MS method found at least a factor of 13 improvement in signal for the drugs investigated. This research provides a potential method for detection of drugs in complex food-based matrices such as baby formula.

Keywords

SPE-DART-MS; Drug Analysis; DART-MS; Baby Formula

1. Introduction

The opioid epidemic continues to be a major concern, responsible for over 400,000 deaths between 1997 and 2017(1). While the victims of this epidemic are predominantly adults, there are a number of issues that translate down to younger generations. In 2012, a National Survey on Drug Use found that approximately 7.5 million children, 17 years and younger, live with at least one parent who abuses drugs(2). Children in these households are more likely to have developmental issues, have less supervision, and have a higher chance of experiencing child abuse or neglect(2). This child abuse and neglect can cause hospitalization or death due to the easy accessibility of drugs being present in the household(3). One such example of this is a study that identified a 2-fold increase in opioid related poisonings between 1997 and 2012 in children and adolescents aged 1 to 19(4). The same study found a 3-fold increase in poisonings associated with children ages 1 to 4, primarily caused by accident(4).

Another common problem facing many babies and young children and their parents is sleeping habits. In a 2011 poll of 26,000 mothers, one in five mothers reported giving their child diphenhydramine to aid in calming their child, and one in twelve mothers gave their children sleep medication(5). This has led to overdoses and sometimes infant deaths(6). Similar incidents have also been reported with daycare workers using over-the-counter medications to help children sleep. In one such instance a woman was sentenced to 8 years in prison for pouring diphenhydramine into baby's milk resulting in the death of an infant (7).

The above examples illustrate an increasing need in the forensic community to detect and identify illicit or prescription drugs in baby formula. Detection of these compounds presents an analytical challenge due to the complex matrix of the baby formula. While there has been significant research into the detection and identification of melamine in baby formula, there is not a large body of work studying narcotics in baby formula. Much of the research in this area has focused on the use of chromatographic-based techniques, such as gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS)(8). Research has shown that direct analysis in real time mass spectrometry (DART-MS) is capable of detecting melamine in powdered milk and milk(9,10). Given the ability of DART-MS to detect trace levels of these compounds in a complex matrix such as milk, and given the well-researched area of drug detection using DART-MS(11–13), the DART-MS platform was chosen to investigate rapid detection of drugs in baby formula.

In this study detection of drugs in baby formula was performed using solid-phase extraction (SPE) coupled with DART-MS. SPE-DART-MS has been demonstrated for the extraction of drugs in urine (14,15) and for the analysis of food-based matrices(16). SPE provides a quick and simple sample prep that allows for faster analysis times and decreases the costs of solvents and disposal. SPE also provides the benefit of improved detection limits compared to traditional analyses(17). In this study, fiber-based SPE was used to extract five drugs (cocaine, dextromethorphan, fentanyl, heroin, and lorazepam) from baby formula. Optimization of the method, establishment of method sensitivities, and comparison to traditional DART-MS analysis was also completed.

2. Materials and Methods

2.1 Materials and Chemicals

The five drugs examined in this study (cocaine, dextromethorphan, fentanyl, heroin, and lorazepam) were purchased as 1.0 mg/mL solutions in methanol or acetonitrile. Cocaine and heroin were purchased from Cayman Chemical (Anne Arbor, MI) while the remaining were purchased from Cerilliant (Round Rock, TX). Working solutions were created from the stock solutions through volumetric dilution with methanol (Honeywell, Charlotte, NC), to concentrations of 10 µg/mL, 1 µg/mL, and 100 ng/mL. Aliquots of these solutions were spiked into the baby formula (Similac Advance, 0-12 months, Instant Formula with Iron, Similac, Columbus, OH) or diluted further in methanol. A solid phase extraction (SPE-it) kit from IonSense (Saugus, MA) with C18 extraction fibers was used for sample extraction. Chromasolv grade water, (Millipore-Sigma, Burlington, MA) methanol (Honeywell), and acetonitrile (Millipore) were used for fiber conditioning.

2.2 Sample Creation and Extraction

Prior to use, the C18 fibers were conditioned by placing them into 2 mL amber glass vials (Restek, Bellefonte, PA) containing 1.0 mL of a 50:50 methanol water mixture. The vials were loaded into the foam insert of the microplate shaker from the SPE-it kit, and the fibers were conditioned for 30 min while shaking at 52.4 rad/s (500 RPM). After conditioning, the fibers were placed in vials containing 1.0 mL of LC-MS Grade water and shaken for approximately 5.0 s at 52.4 rad/s (500 RPM). They were then placed into the sample that was comprised of a methanol, baby formula, drug mixture (a 1.0 mL aliquot of a 50:50 methanol : baby formula mixture spiked with one of the five drugs at the desired concentration) and allowed to extract for 60 minutes while shaking at 52.4 rad/s (500 RPM). For comparison purposes, samples containing just methanol spiked with the drug were also analyzed. Finally, the fibers were again placed into vials containing 1.0 mL of LC-MS Grade water and shaken for approximately 5.0 s at 52.4 rad/s (500 RPM). Fibers were then loaded onto the SPE-it insert for the linear rail of the DART source for analysis. While the fibers in his study were not reused, they can be cleaned by re-conditioning for 30 min while shaking at 52.4 rad/s (500 RPM) in vials containing 1.0 mL of acetonitrile.

2.3 Instrumentation and Sample Analysis

The fibers were analyzed using the SPE holder attached to the linear rail that was mounted to the DART source. A DART-SVP ion source (IonSense) was used for analysis and was coupled with a JMS-T100LP time-of-flight 4G LC-Plus mass spectrometer (JEOL, Peabody, MA). DART parameters that were kept constant throughout the study included the use of helium as the DART ionization gas and an exit grid voltage of +50 V. Unless otherwise stated a gas stream temperature of 350 °C and a linear rail speed of 0.2 mm/s were used. The “12 DIP-it” method within the DART-SVP software was used for controlling the linear rail and had constant parameters of 5 s for the heater wait time and 1 s for the contact closure delay. Mass spectrometer parameters used throughout the study included operation in positive ionization mode, an orifice temperature of 125 °C, an orifice 1 voltage of +20 V, a rings lens voltage of +5 V, an orifice 2 voltage of +5 V, an ion guide RF voltage of 400 V, and a detector voltage of 2300 V. Full scan mass spectra, from *m/z* 60 to *m/z* 700 were collected at 1 scan/s.

3. Results & Discussion

3.1 Method Optimization

The first step of this study was optimizing a method for detection of the drugs in both methanol and baby formula using three of the drugs of interest: cocaine, heroin, and lorazepam. Because there is a significant body of existing work on drug detection by DART-MS(11,18) it was necessary

to optimize only two parameters that may be affected by the implementation of SPE tips and sampling procedures: DART gas stream temperature and linear rail sample speed. Linear rail speed needed to be optimized as it is not a parameter used in traditional DART-MS analysis. The DART gas stream temperature was optimized because use of the linear rail causes the DART to be placed further from the mass spectrometer inlet than with traditional DART analyses, which may affect the temperature experienced by the sample.

The DART gas stream temperature was optimized by evaluating six temperatures: 250 °C, 300 °C, 350 °C, 400 °C, 450°C, and 500 °C at a linear rail speed of 0.2 mm/s. Three replicates of SPE fibers exposed to a 10 µg/mL solution of the individual drugs, in either methanol or baby formula, were analyzed at each temperature. The integrated area from the extracted ion chronographs (EICs) for the base peak of each drug was used for comparison, the results of which are shown in Figure 1.

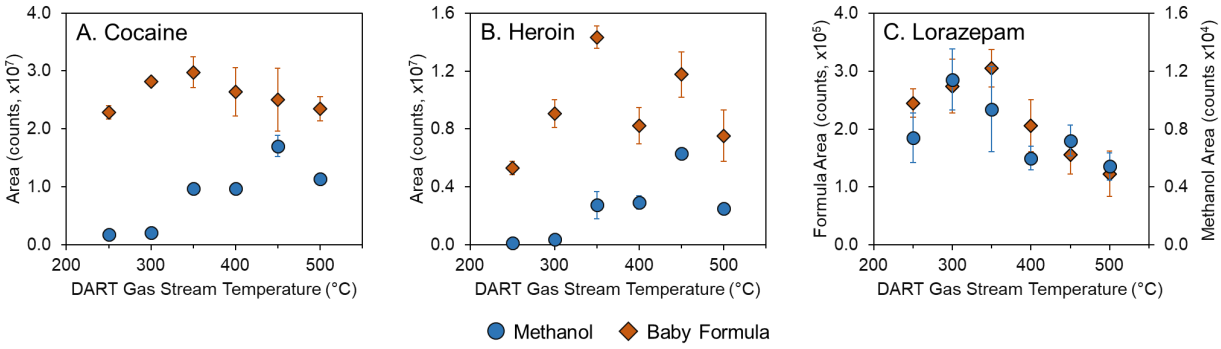

Figure 1. Integrated peak areas of cocaine (A.), heroin (B.), and lorazepam (C.) in methanol (blue circle) and baby formula (orange diamond) as a function of DART gas stream temperature. Error bars show the standard deviation of 3 replicate measurements. Note the separate secondary y-axis for lorazepam in methanol (C.).

The optimization of DART gas stream temperature for drug solutions in both the methanol and baby formula gave varying results. The methanol solutions, which were examined to understand the response independent of the complex matrix baby formula present, produced an optimal response at 450 °C for cocaine and heroin and 300 °C for lorazepam. Interestingly, however, an optimal DART gas stream temperature of 350 °C was found for all three drugs in the presence of the baby formula matrix. The difference in optimal desorption temperature for the two matrices is potentially due to compounds in the baby formula that alter the desorption temperature of the analyte of interest or the co-desorption of other compounds that will either enhance or competitively ionize with the drug. It also appears that lorazepam is less thermally stable than the other two drugs, as a significant decrease in signal was observed at temperatures above 350 °C. Flash desorption or thermal degradation likely also drives the decrease in cocaine and heroin signal above 400 °C.

The second factor that was optimized was the linear rail speed, which controls the amount of time a sample is interrogated by the DART gas stream. Both methanol and baby formula solutions were studied, using a DART gas stream temperature of 450 °C for the methanol solutions and 350 °C for the baby formula solutions. Three rail speeds, the lower (0.2 mm/s), middle (1.5 mm/s), and upper (10 mm/s) bounds of the system were investigated and the integrated peak areas from

the EICs of three replicate measurements were calculated. Replicate measurements were individual tips analyzed in series. The results of this experiment are shown in Figure 2.

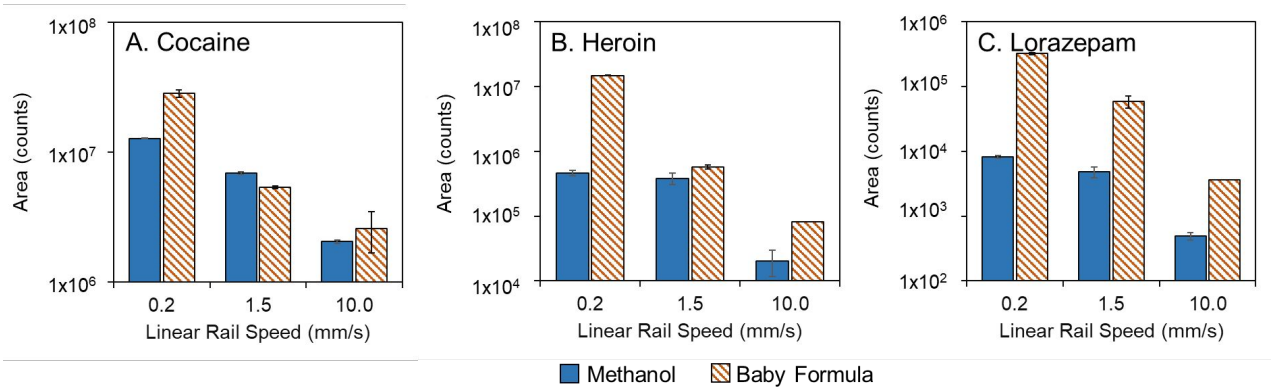

Figure 2. Integrated peak areas of cocaine (A.), heroin (B.), and lorazepam (C.) in methanol (blue) and baby formula (orange striped) as a function of linear rail speed. Error bars show the standard deviation of 3 replicate measurements. Note that peak areas are plotted on a log scale.

For all three drugs studied, regardless of their presence in methanol or baby formula, optimal results were obtained using the slowest rail speed (0.2 mm/s). This was expected as the slower rail speed allows more time for the SPE fibers to interact with the DART gas stream and therefore allows for more thorough desorption of the analytes. The slower rail speed offers a factor of six longer desorption time. The approximate times that the fibers were in the DART gas stream for the three linear rail speeds were 18 s, 3 s, and 1.8 s, for the 0.2 mm/s, 1.5 mm/s, and 10 mm/s speeds, respectively. At faster linear rail speeds, responses from the adjacent SPE fibers began to overlap, which made integration of the peaks and differentiation of spectra from individual fibers difficult. Because of this, for all further experiments a DART gas stream temperature of 350 °C and a linear rail speed of 0.2 mm/s were used.

3.2 Representative Responses & Limits of Detection

After development of the optimized method, all five drugs of interest were investigated to establish their representative responses. Regardless of solution type (methanol or baby formula), all drugs formed protonated molecules, $[M+H]^+$, as expected. No adducts with other cationic species were observed. Comparing the spectral response from a blank SPE filter and one from the pure baby formula (Figures 3A, B) highlights that the SPE extract was able to remove some of the matrix components and it was not selective solely to the drug. The C18 SPE fibers used in this work were able to readily extract all the drugs of interest, as shown in Figure 3C & 3D and Supplement Figures S1 and S2. Without background subtraction, visual identification of the drug signature was difficult at low concentrations, especially for lorazepam (Supplemental Figure S2). Detection at these lower concentrations can be aided by using mass spectral search software or, if possible, spectral subtraction of a clean formula sample. Given that the analytes were present in a complex matrix, only the low voltage in-source collisionally induced dissociation (is-CID) (orifice 1 voltage) setting was investigated. Utilization of higher is-CID voltages, especially those exceeding +40 V, produce noisier spectra due to fragmentation of the drug and matrix components, making detection more difficult.

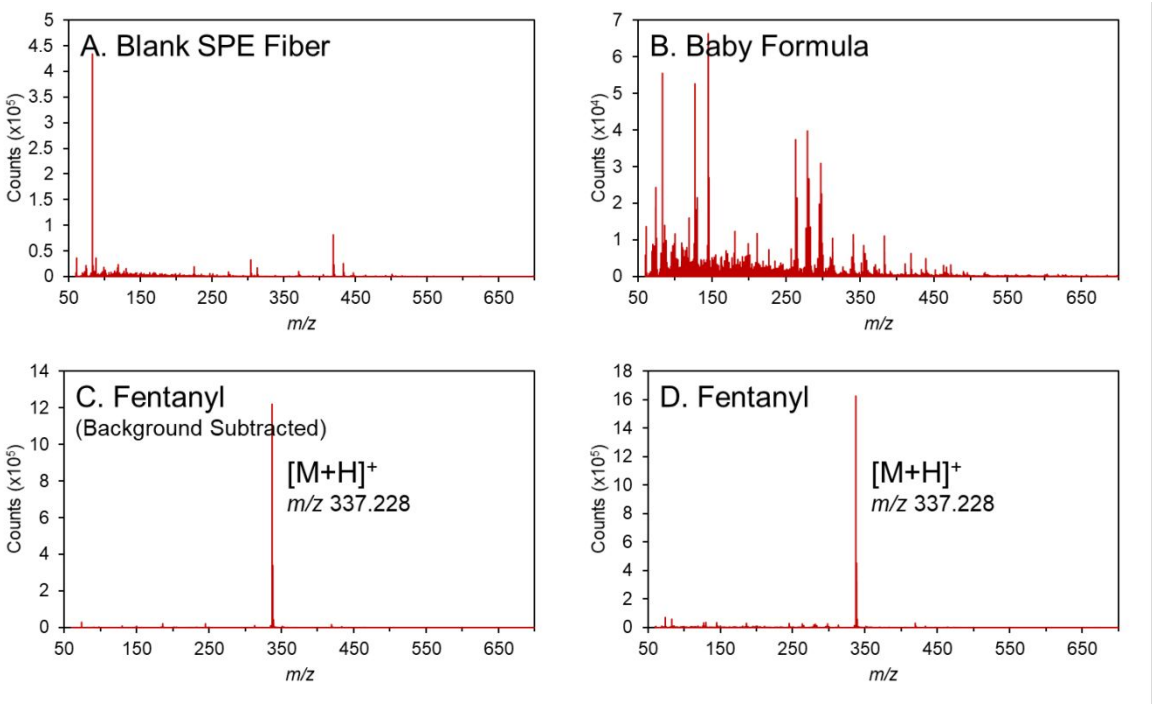

Figure 3. Representative mass spectra of a blank SPE fiber (A.), baby formula (B.), and baby formula doped with 10 µg/mL solution of fentanyl that has been background subtracted (C.), and has not been background subtracted (D.)

Once the representative mass spectral response of the baby formula matrix was established, the approximate sensitives of the method for all five drugs were established. Approximate sensitivities were determined by analyzing the drugs in decreasing concentrations, in triplicate, until a signal to noise ratio (S/N) close to but above 3:1 was obtained. The approximate sensitivities and their respective S/N ratios are shown in Table 1. Cocaine was found to be the most sensitive drug investigated, which was expected given its high ionization efficiency, and is in line with previous drug detection research showing lowest detection limits for cocaine(12,19). Dextromethorphan, which can be added to baby formula through cough syrup, also showed excellent sensitivity. Heroin and lorazepam were the least sensitive compounds, due to either poor extraction, poor desorption, or poor ionization efficiencies. Lower sensitivity for these drugs, compared to the others, has been previously reported(19).

While sensitive detection of the drugs is desired, the method is only practical if the analytes can be detected at levels of toxicological relevance. Table 1 also displays estimated lethal doses for the five drugs in baby formula using published lethal dose data(20–23). The limitations of the availability of data lead to two assumptions for these estimates. First, the estimated lethal dose was calculated using available data for adults. Second, the concentrations reported assume a bottle size of 240 mL as this represents the largest bottle used for feeding infants [16]. Using these estimates, the lethal doses ranged from 41 µg/mL to 5 mg/L, which are two to four orders of magnitude higher than the lowest detectable concentrations. As Table 1 shows, the estimated lethal doses are significantly higher than the approximate sensitivities for the five drugs. The sensitivity of the method shows that detection of non-lethal doses and residues are likely readily achievable using this technique.

Table 1. Base peaks, approximate sensitivities and corresponding signal-to-noise ratios for the five drugs studied as well as estimated lethal doses. Uncertainties represent the standard deviation for triplicate measurements. All estimated lethal doses assume a starting volume of baby formula of 240 mL. These values also assume the lethal dose for adults, since values for infants were not obtainable.

Drug	Base Peak (*m/z*)	Approximate Sensitivity (ng/mL)	Average S/N	Estimated Lethal Dose (ng/mL)	Adult Lethal Dose Reference
Cocaine	304.155 $[M+H]^+$	2.5	7.3 (±1.1)	$5.0x10^6$	(20)
Dextromethorphan	272.201 $[M+H]^+$	5	6.4 (±0.6)	$6.2x10^5$	(21)
Fentanyl	337.228 $[M+H]^+$	10	13.7 (±1.4)	$8.3x10^4$	(22)
Heroin	370.165 $[M+H]^+$	250	14.2 (±1.4)	$2.0x10^5$	(20)
Lorazepam	322.020 $[M+H]^+$	100	12.8 (±3.1)	$4.1x10^4$	(23)

3.3 Signal Suppression/Enhancement

With ambient ionization techniques, like DART-MS, competitive ionization is a common occurrence. Since all analytes are desorbed nearly simultaneously, high ionization efficiency compounds can, at times, complicate detection of analytes of interest that may not be as easily ionized (17). In some instances, ionization of the analyte can be enhanced by other compounds in the matrix. While complete competitive ionization, in which detection of an analyte is not possible, was not observed in this work, the effect of the matrix was still investigated. Aliquots of the methanol and baby formula solutions containing 10 µg/mL of cocaine, heroin, and lorazepam were analyzed in triplicate and the peak areas from the EICs were calculated (Table 2). For all three drugs, a significant enhancement in signal was observed for the formula-containing solutions. This enhancement may be driven by the presence of additional compounds that promote ionization of the drug species.

Table 2. Average peak areas for cocaine, heroin, and lorazepam in baby formula and in methanol. Uncertainties represent the standard deviations of triplicate measurements.

Drug	Peak Area in Baby Formula (Counts)	Peak Area in Methanol (Counts)	Percent Change in Baby Formula
Cocaine	$3.0x10^7$ (±$2.6x10^6$)	$9.7x10^6$ (±$6.9x10^5$)	209 %
Heroin	$1.4x10^7$ (±$7.7x10^5$)	$2.7x10^6$ (±$9.4x10^5$)	418 %
Lorazepam	$3.0x10^5$ (±$3.2x10^4$)	$9.4x10^3$ (±$3.0x10^3$)	3091 %

3.4 Comparison to Traditional DART-MS

The final component of this study compared the SPE-DART-MS to traditional DART-MS analysis to understand the magnitude of enhanced sensitivity offered by the SPE-DART-MS configuration. To evaluate this, analysis of cocaine, heroin, and lorazepam using both the SPE-DART-MS method and an identical DART-MS method was completed. The only difference between the two methods was sample prep-treatment (extraction or not) and sample introduction – where glass microcapillary sampling rods were used for traditional DART-MS. A concentration of 10 µg/mL of the drug in baby formula was analyzed. Both the integrated peak areas (Figure 4 A.) and mass spectral responses were compared. For all three drugs, the response of the drug using SPE-DART-MS compared to conventional DART-MS showed improvements in signal ranging from a factor of 13 for lorazepam to a factor of 118 for heroin. Interpretation of the mass spectra was

also simplified greatly using SPE-DART-MS, as highlighted in Figure 4 B. and 4 C. The use of the SPE fiber was shown to greatly eliminate matrix signal, resulting in the base peak associated with the drug for all spectra.

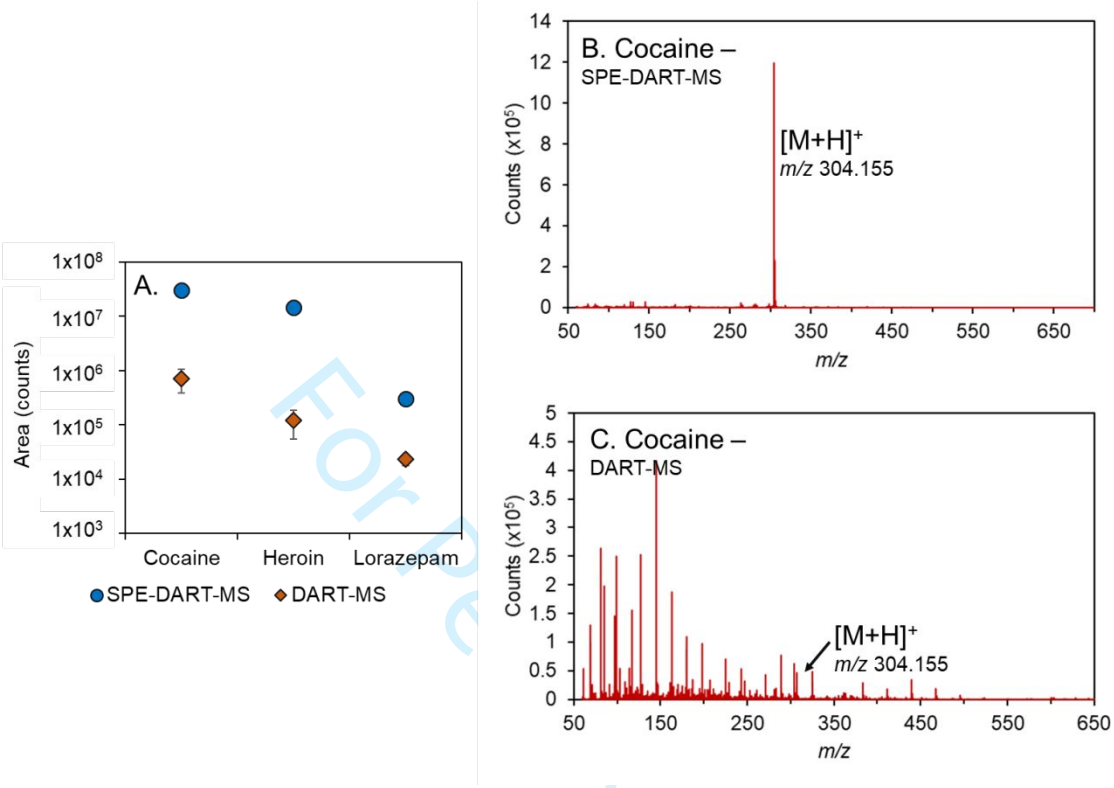

Figure 4. Integrated peak areas of cocaine, heroin, and lorazepam for SPE-DART-MS (blue circle) and traditional DART-MS (orange diamond) (A.). Error bars show the standard deviation of 3 replicate measurements. Note that peak areas are plotted on a log scale. Also shown in a mass spectral comparison of the 10 µg/mL cocaine in baby formula solution when analyzed by SPE-DART-MS (B.) and traditional DART-MS (C.) which have not been background subtracted.

4. Conclusions

SPE-DART-MS provides a sensitive method for the detection of cocaine, dextromethorphan, fentanyl, heroin, and lorazepam in baby formula. Using an optimized method, consisting of a DART gas stream temperature of 350 °C and a linear rail speed of 0.2 mm/s, sensitivities in the range of single to hundreds of ng/mL were obtained. It was observed that the baby formula matrix caused ion enhancement when compared to a methanolic solution of the drug. It was also demonstrated that the SPE-DART-MS provided an enhancement in signal intensity and a more easily interpretable mass spectrum than traditional DART-MS. Utilizing a method such as this would allow chemists to sensitively detect drugs in baby formula in cases where there is suspicion of intentional or unintentional addition of drugs into the formula.

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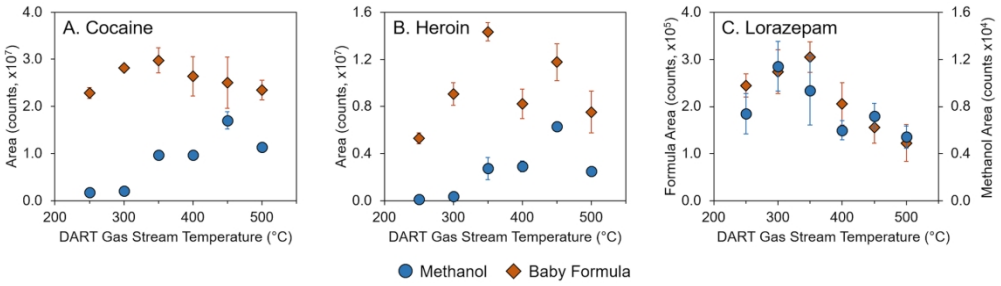

Integrated peak areas of cocaine (A.), heroin (B.), and lorazepam (C.) in methanol (blue circle) and baby formula (orange diamond) as a function of DART gas stream temperature. Error bars show the standard deviation of 3 replicate measurements. Note the separate secondary y-axis for lorazepam in methanol (C.).

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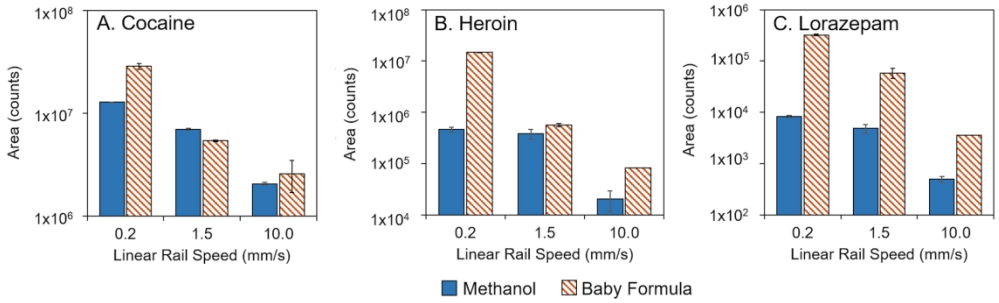

Integrated peak areas of cocaine (A.), heroin (B.), and lorazepam (C.) in methanol (blue) and baby formula (orange striped) as a function of linear rail speed. Error bars show the standard deviation of 3 replicate measurements. Note that peak areas are plotted on a log scale.

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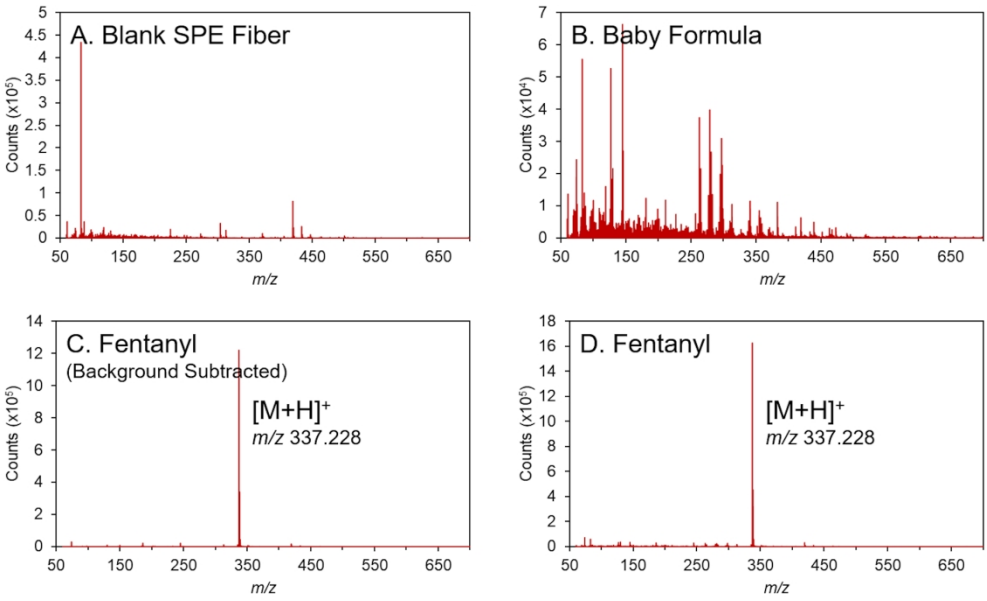

Representative mass spectra of a blank SPE fiber (A.), baby formula (B.), and baby formula doped with 10 µg/mL solution of fentanyl that has been background subtracted (C.), and has not been background subtracted (D.)

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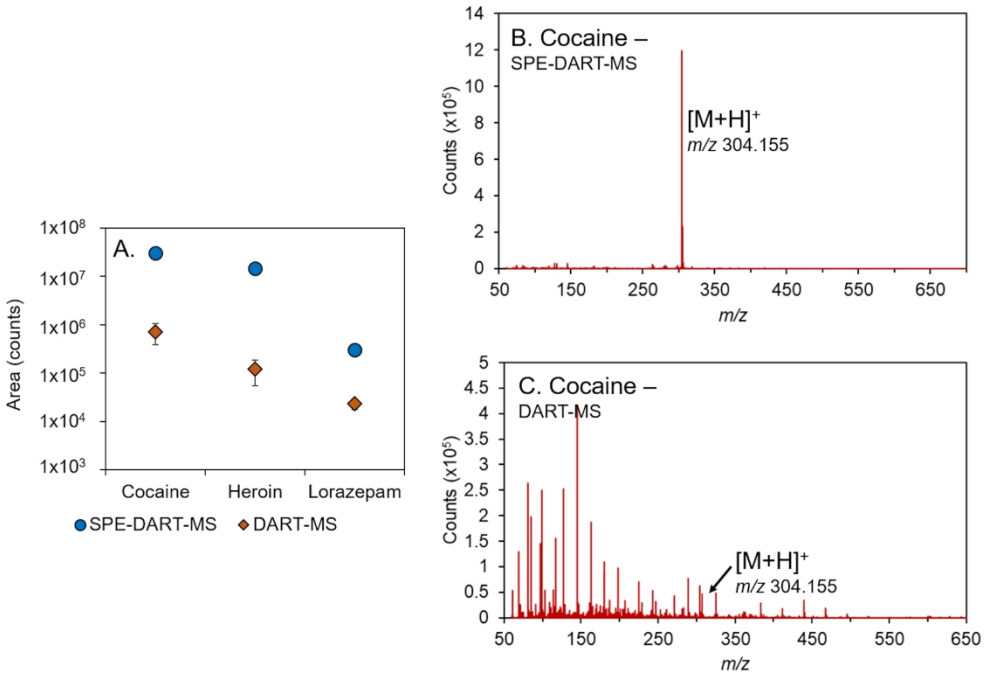

Integrated peak areas of cocaine, heroin, and lorazepam for SPE-DART-MS (blue circle) and traditional DART-MS (orange diamond) (A.). Error bars show the standard deviation of 3 replicate measurements. Note that peak areas are plotted on a log scale. Also shown in a mass spectral comparison of the 10 µg/mL cocaine in baby formula solution when analyzed by SPE-DART-MS (B.) and traditional DART-MS (C.) which have not been background subtracted.

184x127mm (300 x 300 DPI)

Table 1. Base peaks, approximate sensitivities and corresponding signal-to-noise ratios for the five drugs studied as well as estimated lethal doses. Uncertainties represent the standard deviation for triplicate measurements. All estimated lethal doses assume a starting volume of baby formula of 240 mL. These values also assume the lethal dose for adults, since values for infants were not obtainable.

Drug	Base Peak (*m/z*)	Approximate Sensitivity (ng/mL)	Average S/N	Estimated Lethal Dose (ng/mL)	Adult Lethal Dose Reference
Cocaine	304.155 $[M+H]^+$	2.5	7.3 (±1.1)	$5.0x10^6$	(20)
Dextromethorphan	272.201 $[M+H]^+$	5	6.4 (±0.6)	$6.2x10^5$	(21)
Fentanyl	337.228 $[M+H]^+$	10	13.7 (±1.4)	$8.3x10^4$	(22)
Heroin	370.165 $[M+H]^+$	250	14.2 (±1.4)	$2.0x10^5$	(20)
Lorazepam	322.020 $[M+H]^+$	100	12.8 (±3.1)	$4.1x10^4$	(23)

Table 2. Average peak areas for cocaine, heroin, and lorazepam in baby formula and in methanol. Uncertainties represent the standard deviations of triplicate measurements.

Drug	Peak Area in Baby Formula (Counts)	Peak Area in Methanol (Counts)	Percent Change in Baby Formula
Cocaine	3.0×10^7 ($\pm 2.6 \times 10^6$)	9.7×10^6 ($\pm 6.9 \times 10^5$)	209 %
Heroin	1.4×10^7 ($\pm 7.7 \times 10^5$)	2.7×10^6 ($\pm 9.4 \times 10^5$)	418 %
Lorazepam	3.0×10^5 ($\pm 3.2 \times 10^4$)	9.4×10^3 ($\pm 3.0 \times 10^3$)	3091 %

Supplemental Information for:

Detection of Trace Drugs of Abuse in Baby Formula using Solid Phase Extraction Direct Analysis in Real Time Mass Spectrometry (SPE-DART-MS)

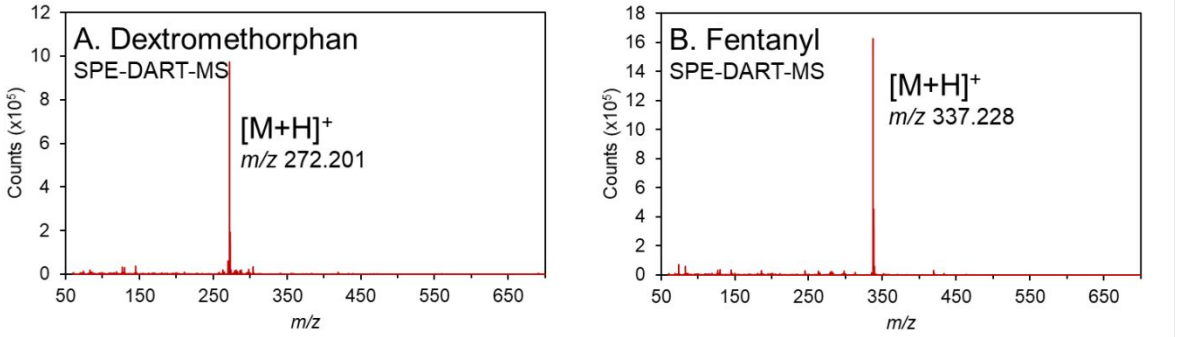

Figure S1. Representative mass spectra of dextromethorphan (A.) and fentanyl (B.) in the presence of baby formula, when analyzed using SPE-DART-MS. The spectra are not background subtracted.

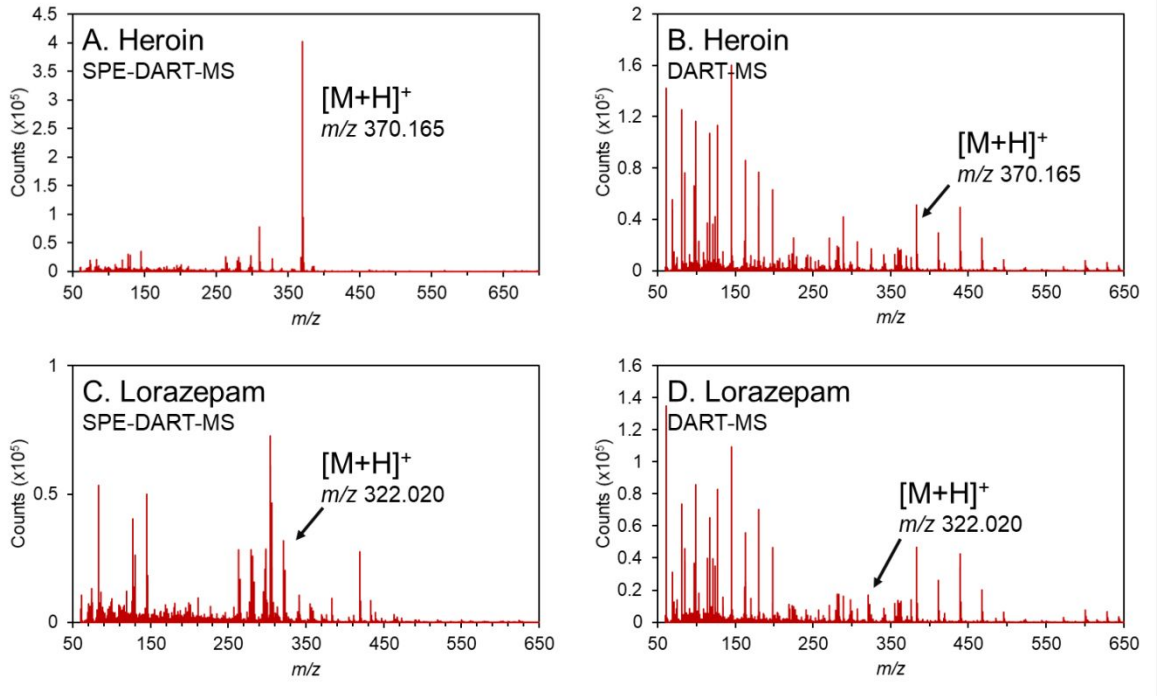

Figure S2. Representative mass spectra of heroin (A. and B.) and lorazepam (C. and D.) in the presence of baby formula, when analyzed using SPE-DART-MS (A. and C.) and traditional DART-MS (B. and D.). The spectra are not background subtracted.