

## Integrated Pressurized Solvent Extraction-Cleanup for the Rapid Determination of Polychlorinated Biphenyls in Meat Samples

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Um método rápido e eficiente, baseado na integração da extração com solvente sob pressão (PSE) com limpeza através do método sólido de extração (SPE) e sistema de cromatografia gasosa com detector por captura de elétrons (GC-ECD) foi desenvolvido para a determinação de bifenilos policlorados (PCBs). Seis indicadores e doze PCBs semelhantes à dioxina foram selecionados como analitos. Este método baseia-se na integração da extração de solvente e de processos de limpeza no interior de uma célula pressurizada de aço inoxidável. As condições de extração otimizadas foram 6 min de extração, a 70 °C e dois ciclos de extração utilizando hexano/cloreto de metileno (1:1, v/v) como solvente de extração. Sílica gel acidificada com ácido sulfúrico foi utilizada para a limpeza integrada do extrato orgânico. Este método permite uma redução significativa no tempo de processo e o consumo de solvente. Repetibilidade, expressa como o desvio padrão relativo, variou de 3 a 6%, e a recuperação para a concentração de 20 ng g<sup>-1</sup> variou entre 71 a 104%. O limite de detecção e quantificação variaram de 0,03 a 0,29 ng g<sup>-1</sup> e 0,1 a 0,97 ng g<sup>-1</sup>, respectivamente. A variação linear foi entre 5 e 100 ng g<sup>-1</sup>. Finalmente, este novo método foi aplicado em amostras de carne de frango e de porco.

A rapid and efficient method based on the integration of pressurized solvent extraction (PSE) with solid phase extraction (SPE) cleanup and gas chromatography system with electron capture detection (GC-ECD) has been developed for determination of polychlorinated biphenyls (PCBs). Six indicators and twelve dioxin-like PCBs were selected as analytes. This method is based on the integration of solvent extraction and cleanup processes inside a pressurized stainless steel cell. The optimum extraction conditions were 6 min of extraction at 70 °C and two cycles of extraction using hexane/methylene chloride (1:1, v/v) as the extraction solvent. Silica gel acidified with sulfuric acid was used for integrated cleanup of the organic extract. This method allows a significant reduction in extraction time and solvent consumption. Repeatability, expressed as the relative standard deviation, ranged from 3 to 6%, and recoveries for concentration of 20 ng g<sup>-1</sup> was ranged between 71 to 104%. The limit of detection and quantification ranged from 0.03 to 0.29 ng g<sup>-1</sup> and 0.1 to 0.97 ng g<sup>-1</sup> respectively. The linear range was between 5 and 100 ng g<sup>-1</sup>. Finally this new method was applied to samples of chicken and pork.

**Keywords:** pressurized solvent extraction, integrated extraction-cleanup, polychlorinated biphenyls, chicken and pork, GC-ECD

### Introduction

Polychlorinated biphenyls (PCBs) (Figure 1) are a group of persistent organic pollutants consisting of more than 200 individual compounds. Even though they have been banned since 1970-1980, they are still found in environmental

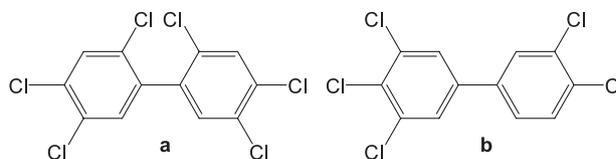
matrices and animal tissue. Their persistence and bioaccumulation make them toxic compounds. Many studies have been conducted to assess the potential exposure of the population to these pollutants through foods. The presence of PCBs in foods of animal origin leads to their absorption in the gut and subsequent incorporation and metabolism in the tissues.<sup>1</sup> The development of new analytical methodologies for rapid determination of these compounds in food matrices

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to enhance control of these pollutants and prevent human health effects is mandatory. Different analytical methods have been proposed for determination of PCBs in foods, such as fruits, meats, fish, eggs, and olive oil.<sup>1-11</sup> As established by Beyer and Biziuk,<sup>12</sup> these methods generally consist of a large number of steps from the extraction of the analyte to its determination, resulting in long pre-treatment times and the consumption of large amounts of organic solvents, which generates considerable waste. There is an increasing need for the development of green sample preparation methods which should be compatible with modern analytical techniques. The conventional approaches to analyze organochlorine compounds in high fat foods involve solvents such as hexane, acetone or methylene chloride for extraction to dissolve the lipids. After the extraction, a laborious cleanup step is usually required.<sup>13</sup>

Modern analytical extraction techniques have been described as alternatives to standard methodologies (soxhlet or ultrasonic extraction). These modern techniques include microwave assisted extraction (MAE)<sup>13,14</sup> and supercritical fluid extraction (SFE) or pressurized liquid extraction (PLE).<sup>5,12,15</sup> PLE uses organic solvents at high temperatures and high pressures to extract compounds from solid.<sup>5,9,16,17</sup> A commercial type of PLE is accelerated solvent extraction (ASE) as developed by Dionex Corporation. The main feature of this system is the equilibrium partitioning of analytes, which occurs in each cycle between the extracting solvent and the matrix. This automated system allows extracting organic compounds from a variety of solid samples. The solvent is used at high temperature and pressure, which increases the availability to solubilize the analyte. On the other hand, it decreases the viscosity of the liquid solvent which allows better penetration of solvent into the matrix.<sup>18</sup> In PLE, a cleanup step can be integrated with the extraction by using an extraction adsorbent mixed with the solid sample, thus avoiding the troublesome steps of conventional sample cleanup and reducing the errors associated with the cleanup step. This integrated approach has been used with food and environmental samples.<sup>5,9,15,16,19,20</sup>

The aim of this work is to develop and validate a rapid and efficient integrated accelerated solvent extraction and solid phase extraction cleanup system for six indicator PCBs (congeners 28, 52, 101, 138, 153, 180) and twelve dioxin-like PCBs (congeners 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189) in chicken and pork samples (Figure 1a and 1b). Samples were ultimately analyzed by gas chromatography system with electron capture detection (GC-ECD) and this new approach was applied to real samples. Only samples that gave positive response were confirmed by gas chromatography tandem mass spectrometry (GC-MS/MS).



**Figure 1.** Molecules of polychlorinated biphenyls (PCBs): (a) 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) indicator and (b) 3,3',4,4',5-pentachlorobiphenyl (PCB-126) dioxin-like.

## Experimental

### Reagents

The standards of PCBs were provided by Dr. Ehrenstorfer Company (Augsburg, Germany). A mixed standard solution of 18 PCBs prepared in hexane was used to spike the samples and for calibration purposes. A standard of hexachlorobenzene provided by Dr. Ehrenstorfer was used as the internal standard at  $1 \mu\text{g mL}^{-1}$  in *n*-hexane. Methylene chloride, acetone, methanol (Merck, Darmstadt, Germany) and *n*-hexane (Fisher Scientific Co., Fair Lawn, NJ, USA), all GC-MS/pesticide grade, were used for the study of solvent extraction. Silica gel 60 (0.063 to 0.200 mm, 70-30 mesh ASTM) (Merck) and neutral alumina (0.063 to 0.200 mm, 70-30 mesh ASTM) (Merck) were activated at 400 °C and Florisil (0.015 to 0.25 mm, 60-100 mesh ASTM) (Fisher) was activated at 650 °C before use. In the case of acid-impregnated silica gel, 50 g of concentrated sulfuric acid (95-97%, Merck) was added to 50 g silica gel 60 (50% m/m). The mixture was shaken to produce a freely flowing solid. Exposure of this mixture to moisture will decrease its effectiveness. Diatomaceous earth, Celite® 545 (Merck) (0.02-0.1 mm), was also used in the cleanup process. Anhydrous sodium sulfate, PA grade (Merck), activated for 16 h at 130 °C, was used as a drying agent.

### Sample preparation and spiking procedure

A spiked dried meat sample was selected for optimization of variables and recovery studies. Meat samples free of PCBs were provided by the Servicio Agrícola y Ganadero (SAG, Santiago, Chile). Chicken and pork (200 g) were frozen at -80 °C for 24 h before lyophilization. The frozen sample was lyophilized with vacuum chamber total pressure and temperature equal to 500  $\mu\text{Hg}$  and -40 °C, respectively, for 72 h. The sample was shredded into small pieces to obtain a larger contact area with the spiked PCBs. This dryness procedure eliminates almost all of the free water contained in the original product, but preserving the molecular structure of the lyophilized substance. Thus the samples remain stable and are easier to store at room temperature.

The dried sample (100 g) was spiked with a standard solution of PCBs at a concentration of 20 ng g<sup>-1</sup> of each congener. The spiking sample was stored in a dry place for two weeks before use.

#### Instrument and apparatus

Lyophilizer L101 (Liobras, São Carlos, Brazil) was used for drying samples of chicken and pork. An ASE 100 extractor (Dionex, Sunnyvale, CA, USA) was used as an integrated extraction/cleanup system for extracting the analytes from the matrices and cleaning up the extract. A Hewlett-Packard system (Palo Alto, USA), consisting of an HP 5890 Series II gas chromatography system with electron capture detection (GC-ECD), was used for identification and quantification of the PCBs studied. A fused silica column (Rtx®-5MS, 5% diphenyl, 95% polydimethylsiloxane: 30 m × 0.25 mm ID × 0.25 μm film thickness), supplied by Restek Corporation (Bellefonte, PA, USA), was employed, with He (99.995%) as carrier gas at flow rate of 1 mL min<sup>-1</sup>. The column temperature was programmed as follows: 100 °C directly to 260 °C at 10 °C min<sup>-1</sup> and holding for 6 min. The total analysis time was 26 min. The injector port was maintained at 250 °C and 1 μL sample volumes were injected in splitless mode.

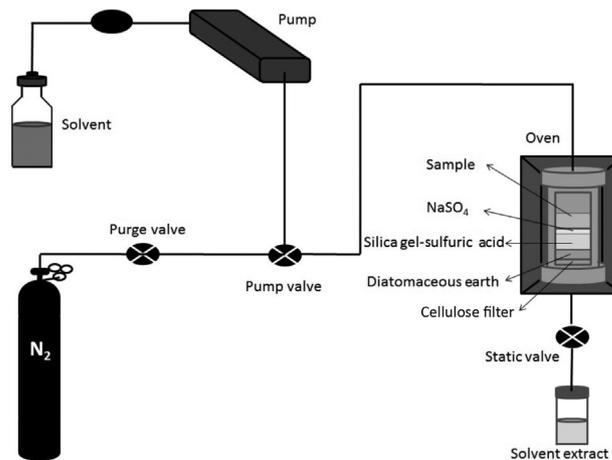
#### Solvent study on PCB extraction

Spiked meat samples (1 g) were weighed and loaded into the 33 mL stainless steel cell of the ASE system. The cell was pressurized to 1500 psi for 10 min at 70 °C, with a volume of approximately 19 mL (60% of flush volume) of the solvent or solvent mixture (1:1, v/v) under study. The cleanup process was performed in a column with 10 g of silica gel acidified with sulfuric acid. The organic phase was concentrated by evaporation with nitrogen gas to a volume of 1 mL, and 1 μL was injected into the GC-ECD.

#### Study of sample cleanup in the integrated system

Traditional adsorbents alumina, silica gel, Florisil® and silica gel acidified with sulfuric acid were tested for the determination of PCBs in pork muscle tissue. The stainless steel cell was loaded with 5 g of each adsorbent in the bottom of the cell, followed by 1 g of anhydrous sodium sulfate (purify by heating to 400 °C for 4 h) and 1 g of spiked dried sample at the top of the cell. The extraction was made with the same pressure, time and temperature conditions of solvent study section. The extract volume was approximately 19 mL of hexane/methylene chloride (1:1, v/v). The eluates were collected and the solvent was evaporated to 1 mL for injection

into the chromatograph (Figure 2). The recovery of target analytes and the efficiency of lipid removal were assessed.



**Figure 2.** Accelerated solvent extraction-solid phase extraction (ASE-SPE) scheme.

#### Optimization of the ASE variables

For the optimization of ASE extractor variables, an experimental design with 3<sup>2</sup> + 3 central points was used, considering the extraction time and extraction cycles as experimental factors. A solvent mixture of hexane/methylene chloride (1:1, v/v) and acidified silica gel were used in all experiments. The temperature was fixed at 70 °C to avoid corrosion of the stainless steel cell.

#### Optimized analytical procedure for real samples

The extraction cell was loaded from the bottom with two cellulose filters, followed by 1 g of diatomaceous earth and 10 g of acidified silica gel. Sodium sulfate (1 g) was then added. The top of the cell was loaded with 1 g of sample (Figure 2). The cell was tightly sealed with the cell cap top and placed in the ASE extractor system. Samples were extracted at 70 °C with hexane/methylene chloride (1:1, v/v) (approximately 19 mL), applying 2 cycles of 6 min each. The final clean organic extract was reduced by a gentle stream of nitrogen to 1 mL prior to injection into the chromatograph.

#### Soxhlet extraction

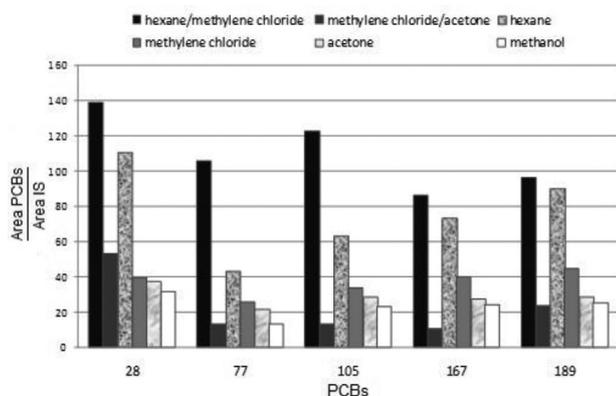
Soxhlet extraction was performed as a comparison methodology. For this comparison, EPA Method 3540C, soxhlet extraction, was used.<sup>21</sup> A portion (5 g) of a meat sample was added to 5 g of sodium sulfate. This mixture was added to a thimble, and the extraction was performed with 300 mL of hexane/methylene chloride (1:1, v/v), for 18 h. The cleanup process was performed in a column with

10 g of silica gel acidified with sulfuric acid. The solvent was removed from the extract in a rotary evaporator at 30 °C under reduced pressure to a final volume of 1 mL prior to injection into the GC-ECD.

## Results and Discussion

### Solvent study for PCB extraction

Different solvents and mixtures of solvents were studied to determine the best extraction efficiency for the 18 compounds studied in the pork sample. Björklund *et al.*,<sup>21</sup> by using this same ASE approach, selected hexane as the extractant for seven PCBs at 100 °C. It was used a temperature of 70 °C because higher temperatures produced corrosion of the cell. Figure 3 shows that, for some representative analytes at 70 °C, a decrease in the solvent polarity increased the extraction efficiency. The best response (area PCB/area IS) was found with a mixture of hexane/methylene chloride (1:1, v/v). Similar behavior was observed by Malavia *et al.*<sup>9</sup> for the PLE extraction of different congeners of polybrominated biphenyls (PBBs) in fish samples. A mixture of hexane/methylene chloride (6:4) at 80 °C was used for the extraction. Gómez-Ariza *et al.*<sup>5</sup> found that a mixture of methylene chloride/pentane (15:85) at 40 °C was optimum to extract PCBs from egg samples. In this study, the proportions of both solvents in the mixture were also tested. Mixtures ranging between 20:80 to 80:20 hexane/methylene chloride were assessed, and the best response was obtained when the hexane proportion was between 25 and 50%. A mixture of 50:50 hexane/methylene chloride was, thus, selected for further studies.

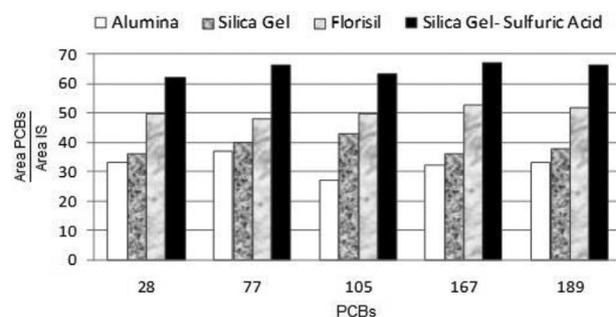


**Figure 3.** Effect of the solvent composition on the extraction (ASE) efficiency of the analytes from pork.

### Solid phase extraction for cleanup

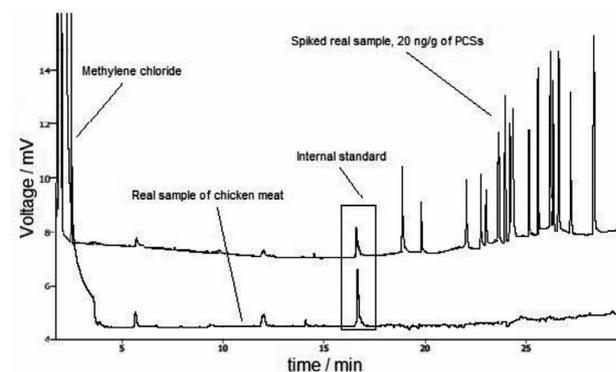
Solid phases such as alumina, silica gel, Florisil® and acidified silica gel were assessed for the integrated

cleanup system. Figure 4 shows that better extraction responses (area PCB/area IS) were found with silica gel 60 impregnated with sulfuric acid, 50% m/m (recovery approximately 67%). Sulfuric acid degrades fat and other organic compounds, but not chlorinated compounds such as PCBs that are highly stable in this medium. The amount of acidified silica gel was also optimized in the range of 5 to 20 g. A quantity of 10 g was selected for further studies. The use of acidified silica gel inside the ASE cell limited the extraction temperature. Extraction temperatures over 70 °C produced corrosion of the ASE cell, as established above.



**Figure 4.** Relative response in the extraction of different PCB compounds with different sorbents for pork.

Under these conditions, the noise on the chromatographic baseline in a real extract was very low (Figure 5 for chicken meat as representative matrix), so the proposed cleanup was sufficient for this type of sample. Extractions at higher temperatures gave rise to a higher co-extraction of lipids, requiring a more complex cleanup process,<sup>20</sup> and risking possible corrosion of the extraction cell.



**Figure 5.** Chromatograms of a real sample of chicken meat and the same sample spiked with PCBs, 20 ng g<sup>-1</sup>.

### Optimization of the PLE procedure

Once the extraction solvent and the solid phase for cleanup were selected, experimental design optimization was performed to obtain the best responses, considering two extraction variables: extraction time and number of

cycles. Extraction temperature was maintained at 70 °C. The use of two extraction cycles of 6 min each was found to be optimal. The addition of one extraction cycle (3 cycles) gave rise to a significant amount of lipids in the final extract, resulting in a lower chromatographic response.

#### Analytical features

To evaluate the performance of the developed method, a meat blank sample was spiked with the 18 PCBs at a level of 20 ng g<sup>-1</sup>. This sample was used to determine the recovery rate for the method. Other analytical features such as a limit of detection (LOD) and precision, relative standard deviation (RSD), were also determined.

The dynamic range studied for the 18 congeners was between 5 and 100 ng g<sup>-1</sup> and 8 points were included in the calibration curve. A good linearity was obtained for all target compounds (correlation coefficients ranging from 0.997 to 0.999). Each point was made by triplicate. The limits of detection and quantification of the method were obtained under these conditions, between 0.03 ng g<sup>-1</sup> (PCB-189) and 0.29 ng g<sup>-1</sup> (PCB-52), defined at a signal to noise ratio (*S/N*) of 3 and 10, respectively<sup>22</sup> (Table 1). The precision of the analytical method calculated by reference to the relative standard deviation (*n* = 6) was between

3.24 and 5.95% (Table 1), which indicated good intra-day precision for the methodology.

The accuracy of the method was calculated based on the percentage of recovery, taking the value of 20 ng g<sup>-1</sup> for a fortified sample to be 100%. Recoveries are between 71 and 104%, indicating a good accuracy for this integrated approach and making it comparable to conventional methods such as soxhlet extraction (Table 1).

When the proposed method is compared with other PLE-SPE integrated procedures for PCBs determination in fat containing samples,<sup>5,15,21</sup> recovery and LOD were similar, but the present method provided a better precision, because a simpler procedure of extraction cell loading is carried out, which also results in a shorter sample preparation procedure. In this case, as shown in Figure 2, the sample is directly loaded after layers of diatomaceous earth, acidic silica and sodium sulfate, whereas in previous works, the sample is pre-mixed with a cleaning phase and then loaded into the extraction cell together with a number of other layers of reagents.

#### Application of proposed method

The integrated method was applied to the determination of the 18 analytes in 20 meat samples of chicken and pork.

**Table 1.** Analytical features of ASE-SPE and soxhlet methods

PCB	R <sup>a</sup>	LOD / (ng g <sup>-1</sup> )	LOQ / (ng g <sup>-1</sup> )	This method (chicken)		Soxhlet (chicken)		This method (pork)		Soxhlet (pork)	
				Recovery / %	RSD / %	Recovery / %	RSD / %	Recovery / %	RSD / %	Recovery / %	RSD / %
28	0.9989	0.26	0.87	82.60	3.24	80.59	4.2	77.02	4.52	79.60	4.8
52	0.9985	0.29	0.97	88.56	4.02	80.45	4.0	67.86	5.60	75.67	4.5
101	0.9994	0.20	0.67	96.06	5.20	87.90	5.9	71.18	5.52	85.45	5.6
81	0.9981	0.21	0.70	88.44	4.30	96.58	4.7	76.83	5.40	95.56	5.0
77	0.9987	0.23	0.77	104.4	4.78	96.69	4.6	98.45	5.83	94.65	4.8
118	0.9994	0.12	0.40	88.22	4.55	95.35	4.0	78.22	4.55	95.10	4.6
123	0.9992	0.07	0.23	83.15	4.79	92.37	5.0	76.46	4.80	91.00	4.9
114	0.9979	0.07	0.23	85.86	4.71	89.41	4.5	78.53	3.81	86.14	4.6
153	0.9984	0.06	0.20	101.6	4.89	98.78	6.3	99.88	5.75	97.25	6.0
105	0.9989	0.08	0.27	96.95	3.66	99.67	4.9	76.79	3.46	99.27	4.5
138	0.9987	0.06	0.20	93.68	3.42	102.80	5.4	75.93	3.76	98.46	5.5
126	0.9988	0.10	0.33	84.03	4.09	87.88	5.4	81.15	3.90	90.78	5.3
167	0.9987	0.05	0.17	97.53	4.55	98.64	4.5	94.93	4.27	102.30	5.0
156	0.9994	0.05	0.17	97.26	4.51	101.56	4.0	93.65	4.43	99.36	4.5
157	0.9986	0.06	0.20	89.27	5.95	88.63	6.9	86.27	5.95	88.40	6.8
180	0.9984	0.03	0.10	98.42	3.90	95.32	4.9	98.42	3.90	91.92	5.0
169	0.9981	0.05	0.17	93.65	5.45	92.66	6.0	89.25	5.49	94.76	6.5
189	0.9983	0.03	0.10	97.23	3.23	100.13	3.6	92.43	5.06	98.14	4.8

<sup>a</sup>Correlation coefficient.

Only one sample gave a positive response to PCB-28 with a concentration of 17 ng of PCBs g<sup>-1</sup> of dry meat. Considering the fat percentage, the concentration in this sample is 43 ng of PCBs g<sup>-1</sup> fat, which exceeds the European regulation.<sup>23</sup> This sample was confirmed by GC-MS/MS which was analyzed in a private laboratory accredited under the ISO/IEC 17025 standard.

A critical comparison of the proposed method with the corresponding standard soxhlet extraction counterpart indicates that the main advantages of the integrated method are as follows: reduced use of sample amount from 5 g to 1 g; reduced use of organic solvent from 300 to 38 mL; reduced human participation; and reduced extraction time from 18 h to 12 min. On the other hand, similar analytical features were observed for the determination of PCBs.

## Conclusions

A selective, rapid and efficient method has been developed for determination of planar (twelve dioxin-like) and non planar (indicators) polychlorinated biphenyls. A mixture of solvents with low polarity (methylene chloride/hexane) turned out to be a better choice for extraction of a group of compounds with differing polarities such as PCBs. The use of acidified silica gel proved to be the best alternative for cleaning up the organic extract obtained from matrices with high lipid content. The recovery values were between 71 and 104%. The precision of the method, calculated in terms of the coefficient of variation, was between 3.2 and 5.9%, allowing us to conclude that an integrated approach to extraction/cleanup provides a quantitative method with very good repeatability, comparable to standard methodologies. Poor handling of the sample and the organic extract would cause a considerable reduction in loss of sample and analytes.

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