

# APPLICATIONS

## Extraction and Quantification of Parabens from Baby Wash and Shampoo Using Novum<sup>TM</sup> Simplified Liquid Extraction (SLE) and LC/UV

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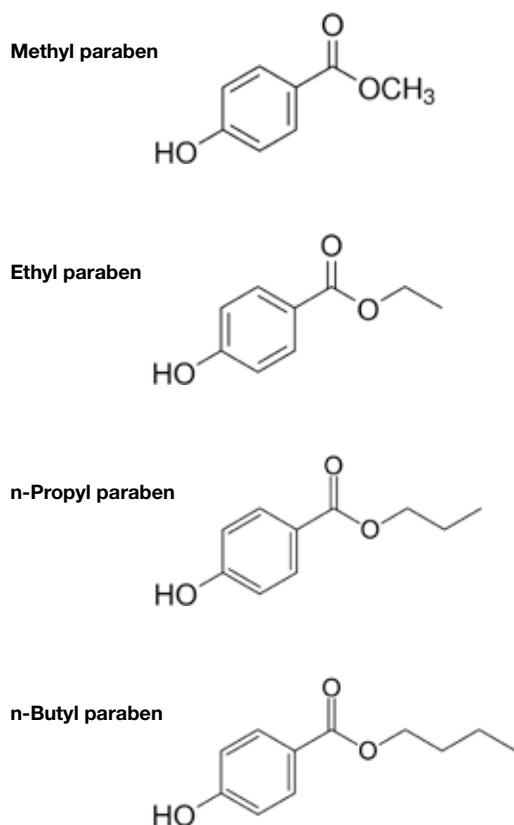
**Matt Brusius**  
Product Manager,  
Sample Preparation  
Matt Brusius is an avid ice hockey player. He likes skating backwards and taking slapshots from the point.

### Introduction

Parabens (esters of para-hydroxy benzoic acid) are widely used as food additives and synthetic preservatives in cosmetics and personal care products as well as in pharmaceutical formulations. Common types include methyl paraben, ethyl paraben, n-propyl paraben, and n-butyl paraben (**Figure 1**). Parabens are also used in combination with other preservatives to safeguard against an extensive range of microorganisms. It has been disputed that parabens can also be linked to breast cancer and other reproductive issues which have led to an onset of studies to determine the role that parabens play in diseases. It is therefore of the utmost importance to have a quantitative method that can accurately monitor paraben levels in food and other personal care products. In this technical note we describe a simple extraction procedure to quantitate four of the most commonly used parabens from baby wash using Novum Simplified Liquid Extraction (SLE) products in conjunction with a Kinetex<sup>®</sup> EVO C18 5  $\mu$ m core-shell HPLC column.

### Experimental Conditions

**Figure 1.** Analyte structures



### Sample Preparation

Novum SLE 12 cc Tube, part no. 8B-S138-KDG

#### Pretreatment

1. Add 1.25 mL acetone and 0.6 mL 1 % NaCl solution to  $0.5 \pm 0.05$  g of a commercial baby wash/shampoo which has been spiked to contain 100  $\mu$ g/g parabens
2. Gently mix for 30 seconds, avoid excessive foaming/emulsions
3. Wait 30 seconds until the emulsion dissipates
4. Centrifuge for 10 minutes at 3000 rpm
5. Collect the supernatant and proceed to SLE procedure (below)

#### SLE Procedure

##### Sample Loading

- Load the sample from pretreatment step onto the Novum SLE sorbent and apply a short and gentle pulse of vacuum (~ 5" of Hg for 5-10 seconds or until the sample has completely entered the sorbent)
- Wait for 5 minutes

##### Elution

- Dispense 10 mL of ethyl acetate onto the SLE media and allow the solvent to elute by gravity for 10 minutes and collect the eluent
- Apply vacuum at 5-10" of Hg to complete the extraction

*NOTE:* Prolonged application of vacuum will result in elution of baby wash out of the SLE media and into the final extracted solvent

##### Reconstitute

- Evaporate the final extract to complete dryness under slow stream of  $N_2$  at 45  $^{\circ}C$
- Reconstitute the dry residue in 0.5 mL of methanol
- Centrifuge for 10 minutes at 6000 rpm
- Collect supernatant and inject onto the HPLC

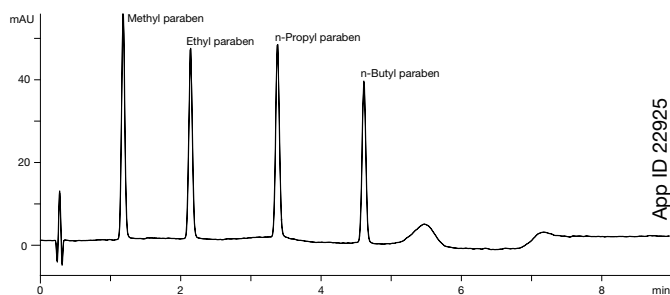
### HPLC Conditions

<b>Column:</b>	Kinetex <sup>®</sup> 5 $\mu$ m EVO C18										
<b>Dimensions:</b>	100 x 3.0 mm										
<b>Part No.:</b>	00D-4633-YO										
<b>Mobile Phase:</b>	A: 10 % Acetonitrile in Water B: Acetonitrile										
<b>Gradient:</b>	<table border="0"> <tr> <td><b>Time (min)</b></td> <td><b>B (%)</b></td> </tr> <tr> <td>0</td> <td>10</td> </tr> <tr> <td>4</td> <td>30</td> </tr> <tr> <td>5</td> <td>40</td> </tr> <tr> <td>6</td> <td>40</td> </tr> </table>	<b>Time (min)</b>	<b>B (%)</b>	0	10	4	30	5	40	6	40
<b>Time (min)</b>	<b>B (%)</b>										
0	10										
4	30										
5	40										
6	40										
<b>Flow Rate:</b>	1.5 mL/min										
<b>Injection Volume:</b>	2 $\mu$ L										
<b>Temperature:</b>	Ambient										
<b>Detection:</b>	UV @ 254 nm										
<b>System:</b>	Agilent <sup>®</sup> 1100 Binary Pumps										

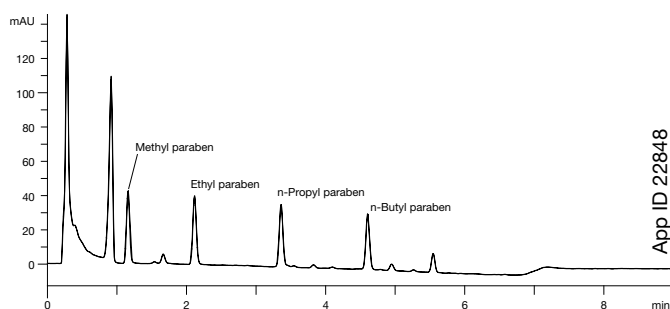


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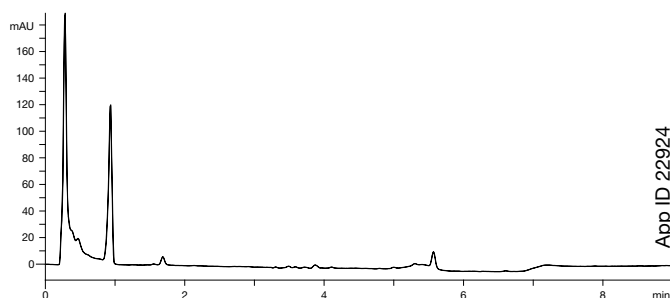
**Figure 2.** Representative reference chromatogram of a neat sample spiked at 100 µg/g



**Figure 3.** Representative chromatogram of paraben spiked baby wash extracted from Novum™ SLE 12 cc Tube



**Figure 4.** Representative chromatogram of blank baby wash extracted from Novum SLE 12 cc Tube



## Results

**Table 1.** % Absolute recovery of parabens extracted from baby wash using a Novum SLE 12 cc tube

Analyte	Absolute Recovery (%)	%CV(N=8)
Methyl paraben	85	7.2
Ethyl paraben	88	5.9
n-Propyl paraben	82	6.0
n-Butyl paraben	86	5.0

## Results and Discussion

### Pretreatment

Acetone was added to the solution to decrease the surface tension of the sample thus reducing the surfactant effect of the matrix contaminants. Furthermore, the reduction in viscosity improves the sample handling and loading. The addition of sodium chloride was deemed necessary to increase the ionic strength of the solution and by extension enhance the recovery of the hydrophobic analytes from the detergent mixture.

### Extraction Solvent

Ethyl acetate was chosen as the extraction solvent for the SLE procedure as it produced excellent levels of cleanliness which is shown in **Figure 3** and also resulted in recovery values that were greater than 80 % (**Table 1**).

### Extraction Solvent

**Figure 2** shows a chromatogram of the neat sample spiked at known concentrations for all four parabens—methyl, ethyl, n-propyl, and n-butyl. The peak area corresponds to 100 % recovery and is the reference for calculating **absolute recovery**. **Figure 3** is a chromatogram of the pre-spiked sample. By comparing this peak area to the peaks in the reference chromatogram (**Figure 2**), the percent recovery is calculated and shown in **Table 1**. All parabens were spiked at 100 µg/g. **Figure 4** shows the blank sample, which is baby wash that has gone through the entire SLE extraction process with no addition of paraben standards. It is used as a control in this experiment and shows that none of the peaks that were present in the unspiked sample coeluted with the analytes of interest so that they can be accurately quantitated.

## Conclusion

By using ethyl acetate as the extraction solvent, the resulting eluent displayed sufficient cleanliness and greater than 80 % recovery for all four parabens with excellent precision (% RSDs < 8 %). The method used for the SLE extraction is very simple when compared to traditional liquid-liquid extraction, indicating that SLE using Novum SLE products can provide clean, accurate results when extracting parabens from a shampoo and baby wash matrix that can save steps as compared to a liquid-liquid extraction.

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## Ordering Information

### Novum™ Simplified Liquid Extraction (SLE) 96-Well Plates

Part No.	Description	Unit
8E-S138-FGA	Novum SLE MINI 96-Well Plate	1/pk
8E-S138-5GA	Novum SLE MAX 96-Well Plate	1/pk



### 96-Well Plate Accessories

Part No.	Description	Unit
<b>Collection Plates (deep well, polypropylene)</b>		
AH0-7192	96-Well Collection Plate, 350 µL/well	50/pk
AH0-7193	96-Well Collection Plate, 1 mL/well	50/pk
AH0-7194	96-Well Collection Plate, 2 mL/well	50/pk
AH0-8635	96-Well Collection Plate, 2 mL/well Square/Round-Conical	50/pk
AH0-8636	96-Well Collection Plate, 2 mL/well Round/Round, 8 mm	50/pk
AH0-7279	96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk
<b>Sealing Mats</b>		
AH0-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk
AH0-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk
AH0-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk
AH0-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk
AH0-8633	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk
AH0-8634	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk
AH0-7362	Sealing Tape Pad	10/pk
<b>Vacuum Manifold</b>		
AH0-8950	96-Well Plate Manifold, Universal with Vacuum Gauge	ea

### Novum SLE Tubes

Part No.	Description	Unit
8B-S138-FAK	Novum SLE 1 cc tubes	100/pk
8B-S138-5BJ	Novum SLE 3 cc tubes	50/pk
8B-S138-JCH	Novum SLE 6 cc tubes	30/pk
8B-S138-KDG	Novum SLE 12 cc tubes	20/pk



### Tube Accessories

<b>Vacuum Manifolds</b>		
Part No.	Description	Unit
AH0-6023	12-Position Vacuum Manifold Set	ea
AH0-6024	24-Position Vacuum Manifold Set	ea

**guarantee**

If Novum SLE products do not perform as well or better than your current SLE product, return the product for a full refund.



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Novum is patent pending

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