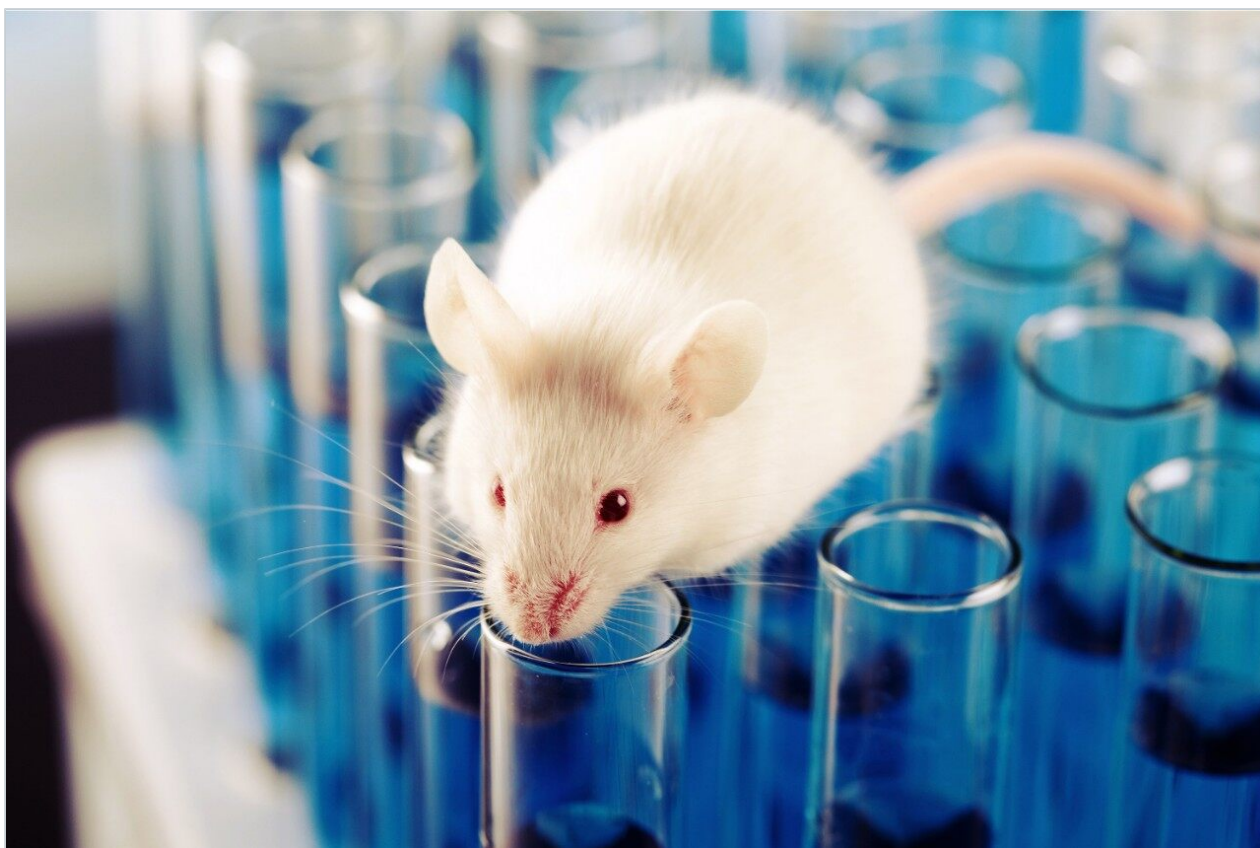


## Niflumic Acid/Camphorsulfonic Acid Extracted from Rat Plasma

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Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

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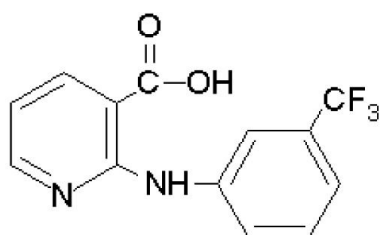
### Abstract

This application brief demonstrates analysis of niflumic acid/camphor sulfonic acid extracted from rat plasma.

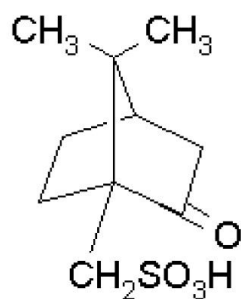
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## Introduction

The compounds used in this study are - Niflumic Acid and (1S)-Camphor-10-sulfonic acid.



**Niflumic Acid - 115% recovery**  
**MW 282.2, C<sub>13</sub>H<sub>9</sub>F<sub>3</sub>N<sub>2</sub>O<sub>2</sub>**



**(1S)-Camphor-10-sulfonic acid -**  
**109% recovery**  
**MW 232.3, C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>S**

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## Experimental

## Oasis WAX SPE Protocol

|              |                                                                                         |
|--------------|-----------------------------------------------------------------------------------------|
| Plate:       | Oasis WAX $\mu$ Elution Plate                                                           |
| Condition:   | 200 $\mu$ L MeOH                                                                        |
| Equilibrate: | 200 $\mu$ L H <sub>2</sub> O                                                            |
| Load:        | 100 $\mu$ L 1:1 diluted rat plasma, 10 pg/ $\mu$ L, w/2% H <sub>3</sub> PO <sub>4</sub> |
| Wash 1:      | 200 $\mu$ L 25 mM NH <sub>4</sub> CH <sub>2</sub> COOH buffer in H <sub>2</sub> O, pH 5 |
| Wash 2:      | 200 $\mu$ L MeOH                                                                        |
| Elute:       | 50 $\mu$ L (25 $\mu$ L x 2) MeOH w/2% NH <sub>4</sub> OH                                |
| Dilute:      | 50 $\mu$ L H <sub>2</sub> O with 2% HCOOH                                               |
| Inject:      | 10 $\mu$ L                                                                              |

## Conditions

|                   |                                                                          |
|-------------------|--------------------------------------------------------------------------|
| Column:           | SunFire C <sub>18</sub> 2.1 x 20 mm <i>IS</i> , 3.5 $\mu$ m              |
| Part number:      | 186002531                                                                |
| Mobile phase A:   | H <sub>2</sub> O with 10 mM NH <sub>4</sub> CH <sub>3</sub> COOH, pH 5.5 |
| Mobile phase B:   | MeOH with 10 mM NH <sub>4</sub> CH <sub>3</sub> COOH, pH 5.5             |
| Flow rate:        | 0.4 mL/min                                                               |
| Injection volume: | 10 $\mu$ L                                                               |

|                       |                                                            |
|-----------------------|------------------------------------------------------------|
| Sample concentration: | 10 pg/μL each analyte                                      |
| Temperature:          | Ambient                                                    |
| Instrument:           | Waters Alliance 2695<br>Waters Micromass Quattro micro API |
| ESI source temp.:     | 150 °C                                                     |
| Desolvation temp.:    | 350 °C                                                     |
| Cone gas flow:        | 50 L/Hr                                                    |
| Desolvation gas flow: | 600 L/Hr                                                   |
| Collision Cell:       | 1.23e <sup>-3</sup> bar (Ar gas)                           |
| Compounds             | MRM Transition                                             |
| Niflumic Acid         | <i>m/z</i> 281.1 → 237.1                                   |
| Camphorsulfonic Acid  | <i>m/z</i> 231.1 → 79.9                                    |

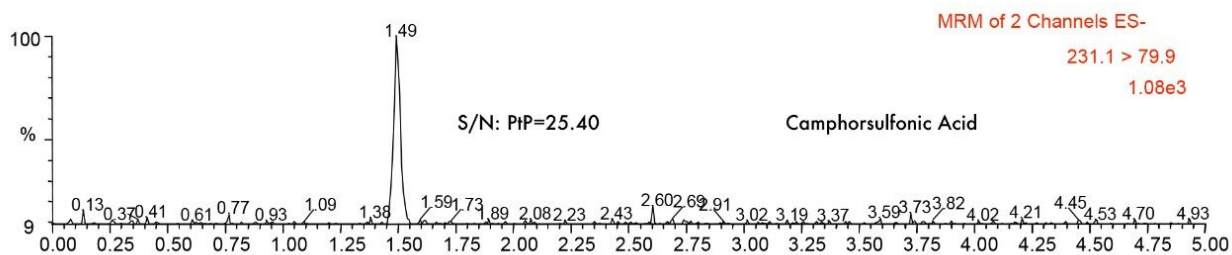
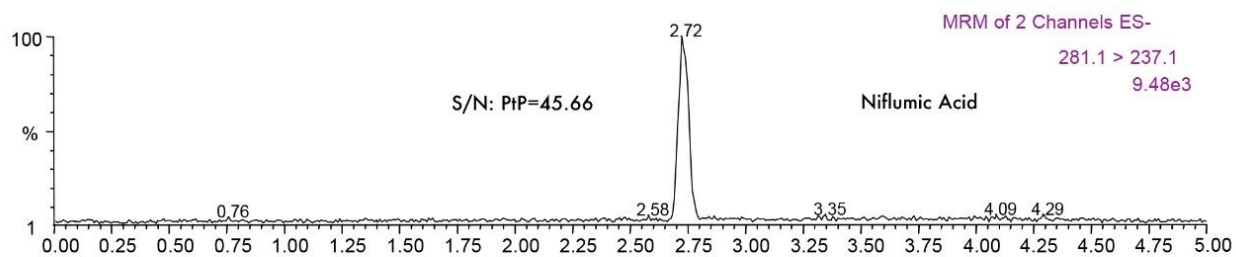
## Gradient

| Time<br>(min) | Profile |    |
|---------------|---------|----|
|               | %A      | %B |
| 0.0           | 95      | 5  |
| 3.0           | 5       | 95 |
| 4.0           | 5       | 95 |
| 4.1           | 95      | 5  |
| 5.0           | 95      | 5  |

---

## Results and Discussion

|                      | MRM transition                  | Cone<br>(V) | CID<br>(eV) |
|----------------------|---------------------------------|-------------|-------------|
| Niflumic acid        | $m/z$ 281.1 $\rightarrow$ 237.1 | 30          | 17          |
| Camphorsulfonic acid | $m/z$ 231.1 $\rightarrow$ 79.9  | 45          | 30          |



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Alliance HPLC System <<https://www.waters.com/534293>>

WA41895, May 2005