Analytical report

Manual and robotic preparation of Levothyroxine Sodium Tablets
## Contents

I. Report 3

1. Introduction 4

2. Conclusion 4

3. Method and preparation 5
   3.1. Chromatographic parameters 5
       3.1.1. Gradient Program 5
       3.1.2. Solvent preparation 6
   3.2. Standard and Sample Preparation 7
       3.2.1. Working standard Preparation 7
       3.2.2. Manual sample preparation for 0.025mg Strength 7
       3.2.3. accroma™ 1 sample preparation for 0.025mg Strength 7
       3.2.4. accroma™ 2 sample preparation for 0.025mg Strength 8
       3.2.5. accroma™ 3 sample preparation for 0.025mg Strength 8
       3.2.6. accroma™ 4 sample preparation for 0.025mg Strength 9
       3.2.7. Impression of accroma™ working 9

4. Results and comparison 10
   4.1. HPLC Results 10
   4.2. Working standard 11
   4.3. Manual sample preparation 12
   4.4. accroma™ sample 1 13
   4.5. accroma™ sample 2 14
   4.6. accroma™ sample 3 15
   4.7. accroma™ sample 4 16
   4.8. accroma™ Log files 17
   4.9. Comparison 17

5. Discussion 19
II. Appendix  

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Literature</td>
<td></td>
</tr>
<tr>
<td>A.1.</td>
<td>Assay</td>
</tr>
<tr>
<td>A.</td>
<td></td>
</tr>
<tr>
<td>B. Chromatograms</td>
<td></td>
</tr>
<tr>
<td>B.1.</td>
<td>Chromatograms of the working standard</td>
</tr>
<tr>
<td>B.2.</td>
<td>Chromatograms of the manual sample preparation</td>
</tr>
<tr>
<td>B.3.</td>
<td>Chromatograms of the accroma\textsuperscript{TM} 1 sample preparation</td>
</tr>
<tr>
<td>B.4.</td>
<td>Chromatograms of the accroma\textsuperscript{TM} 2 sample preparation</td>
</tr>
<tr>
<td>B.5.</td>
<td>Chromatograms of the accroma\textsuperscript{TM} 3 sample preparation</td>
</tr>
<tr>
<td>B.6.</td>
<td>Chromatograms of the accroma\textsuperscript{TM} 4 sample preparation</td>
</tr>
<tr>
<td>C. Sample log files</td>
<td></td>
</tr>
<tr>
<td>C.1.</td>
<td>Sample log accroma\textsuperscript{TM} 1</td>
</tr>
<tr>
<td>C.2.</td>
<td>Sample log accroma\textsuperscript{TM} 2</td>
</tr>
<tr>
<td>C.3.</td>
<td>Sample log accroma\textsuperscript{TM} 3</td>
</tr>
<tr>
<td>C.4.</td>
<td>Sample log accroma\textsuperscript{TM} 4</td>
</tr>
</tbody>
</table>
Part I.

Report
1. Introduction

The purpose of this study was to compare the manual method of preparation of Levothyroxine Sodium Tablets (25 µg) for uniformity of content test with a newly developed robotic preparation using the accroma™ robot. Test methods used and full analytical experimental details are provided in this report. For consistency all tablets used in this study came from the same batch N22HA18001 and an analytical standard was provided (purity 90.3%) for chromatographic analysis.

2. Conclusion

Extraction times by the accroma™ robot were shown to be significantly shorter than by the manual method of ultrasonication (3 mins vs 30 mins for accroma™ robot and a manually prepared sample respectively). Not only was full extraction achieved in 3 minutes by the accroma™ robot but in addition a slightly greater recovery was achieved when compared to the manual method. When considering the overall time of preparing a single sample from weighing to transferring the HPLC vial into the liquid chromatograph ready for chromatographic analysis a significant reduction in sample preparation time was observed when preparing sample by the accroma™ robot 14 mins compared to 45 minutes by manual means. When considering multiple samples are usually prepared for uniformity of content (10 tablets are usually taken) and often multiple batches require analysis the accroma™ robot offers a very significant cost saving by significantly reducing sample preparation time. Also as the accroma™ robot is fully integrated to HPLC there is no need for manual intervention once the sample has been placed in the robots extraction tube. Other considerations are the audit data trail that is available from the accroma™ robot and how this increases data integrity and compliance. Throughout this study no degradation of samples or adsorption was observed for any of the samples.
3. Method and preparation

The chromatographic conditions used to analyse all amples is given below in section 3.1 and details of all standard and sample preparations given in section 3.2. Full details of the manual sample preparation can be found in Appendix A.1.

3.1. Chromatographic parameters

Instrument: HPLC equipped with UV detector
Column: Zorbax Extend C18, (150 x 4.6) mm, 5 µm
Column oven temperature: 40°C
Flow rate: 1.5 ml/min
Injection volume: 50 µL
Detection (UV Detector): 225 nm
Run time: Refer gradient program
Retention time for Active peak: Between 3.0 and 4.5 minutes

3.1.1. Gradient Program

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>Mobile Phase A [%]</th>
<th>Mobile Phase B [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>4.40</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>4.50</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>5.60</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>5.70</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>8.00</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1.: Gradient program
3.1.2. Solvent preparation

Buffer
Bring distilled water to pH 6.50 with ready to use phosphate buffer solution.

Mobile phase A
Homogeneous solution of the following solvents:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>700</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>300</td>
</tr>
</tbody>
</table>

Mobile phase B
Homogeneous solution of the following solvents:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>500</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>500</td>
</tr>
</tbody>
</table>

Diluent
Homogeneous solution of the following solvents:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>600</td>
</tr>
<tr>
<td>Water</td>
<td>400</td>
</tr>
<tr>
<td>Orthophosphoric acid 85%</td>
<td>0.5</td>
</tr>
</tbody>
</table>
3.2. Standard and Sample Preparation

3.2.1. Working standard Preparation

Accurately weighed and transferred 26.2mg of working standard (Purity: 90.3%) into 100ml volumetric flask. Make up the volume to the mark with methanol sonicate to dissolve completely. Transfer 1ml of this solution into a 50ml volumetric flask and make up the volume to the mark with diluent.

(Concentration with considered purity: 0.0047mg/ml)

3.2.2. Manual sample preparation for 0.025mg Strength

Randomly select 20 tablets. Accurately weigh and transfer the tablets into a 100ml volumetric flask and add 100ml of diluent. Sonicate for 30 minutes with intermittent shaking after every 5 minutes. Filter the suspension through 0.45µ PVDF syringe filter discarding 3ml of filtrate.

(Concentration: 0.0050mg/ml)

3.2.3. accroma™ 1 sample preparation for 0.025mg Strength

Put two steel balls in an extraction tube, close and put it on the rack of the accroma™. Start the previously programmed Tara weighing workflow. Randomly select 20 tablets and transfer the tablets into the tared extraction tube. Start the created workflow, which will weigh the tablets and then start the sample preparation. The accroma™ dry mills the tablets for 5min, adds 100ml of diluent and shakes the sample for another 10min. After the extraction time the accroma™ will filter the suspension through 0.45µ PVDF syringe filter discarding 3ml of filtrate. Samples are filtered in glass HPLC vials which are closed by the accroma™ robot and placed on the tray ready for collection for chromatographic.

(Concentration: 0.0050mg/ml)
3.2.4. accroma™ 2 sample preparation for 0.025mg Strength

Put two steel balls in an extraction tube, close and put it on the rack of the accroma™. Start the previously programmed Tara weighing workflow. Randomly select 20 tablets and transfer the tablets into the tared extraction tube. Start the created workflow, which will weigh the tablets and then start the sample preparation. The accroma™ dry mills the tablets for 1min, adds 100ml of diluent and shakes the sample for another 5min. After the extraction time the accroma™ will filter the suspension through 0.45µm PVDF syringe filter discarding 3ml of filtrate. Samples are filtered in glass HPLC vials which are closed by the accroma™ robot and placed on the tray ready for collection for chromatographic.

(Concentration: 0.0050mg/ml)

3.2.5. accroma™ 3 sample preparation for 0.025mg Strength

In test sample 3 we were interested in determining the recovery following extraction into neat methanol and then dilution with diluent. For this one experiment methanol was added to the extraction tube manually. This could have been performed by the robot but on this occasion the solvent was added manually. Put two steel balls in an extraction tube, close and put it on the rack of the accroma™. Start the previously programmed Tara weighing workflow. Randomly select 20 tablets and transfer the tablets into the tared extraction tube. Start the created workflow, which will weigh the tablets and then start the sample preparation. The accroma™ dry mills the tablets for 1min and puts the sample back on the rack. Manually add 20ml of methanol. The robot will continue to shake the sample for 2min, then add 80ml of Diluent and shake for another 2min. After the extraction time the accroma™ will filter the suspension through 0.45µm PVDF syringe filter discarding 3ml of filtrate. Samples are filtered in glass HPLC vials which are closed by the accroma™ robot and placed on the tray ready for collection for chromatographic.

(Concentration: 0.0050mg/ml)
3.2.6. accroma™ 4 sample preparation for 0.025mg Strength

Put two steel balls in an extraction tube, close and put it on the rack of the accroma™. Start the previously programmed Tara weighing workflow. Randomly select 20 tablets and transfer the tablets into the tared extraction tube. Start the created workflow, which will weigh the tablets and then start the sample preparation.

The accroma™ dry mills the tablets for 0.5min, adds 100ml of diluent and shakes the sample for another 2.5min. After the extraction time the accroma™ will filter the suspension through 0.45µ PVDF syringe filter discarding 3ml of filtrate. Samples are filtered in glass HPLC vials which are closed by the accroma™ robot and placed on the tray ready for collection for chromatographic.

(Concentration: 0.0050mg/ml)

3.2.7. Impression of accroma™ working

The figure 1 below shows the accroma™ holding an extraction tube containing the sample suspension.

Figure 1.: accroma™ with sample
4. Results and comparison

4.1. HPLC Results

The table 2 below shows the sequence of the HPLC analysis.

<table>
<thead>
<tr>
<th>Order</th>
<th>Injections</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Working standard</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Manual</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>accroma\textsuperscript{TM} 1</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Working standard</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>accroma\textsuperscript{TM} 2</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>accroma\textsuperscript{TM} 3</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>Working standard</td>
</tr>
</tbody>
</table>

Table 2.: Sequence 1

Based on the results observed for the 3 accroma\textsuperscript{TM} prepared samples it was decided to complete a 4\textsuperscript{th} sample preparation to see if a 2 minute extraction time was possible. The table 3 below shows the sequence used for this 4\textsuperscript{th} accroma\textsuperscript{TM} preparation method.

<table>
<thead>
<tr>
<th>Order</th>
<th>Injections</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>Working standard</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>accroma\textsuperscript{TM} 4</td>
</tr>
</tbody>
</table>

Table 3.: Sequence 2

Section 4.2 to 4.7 show example chromatograms of the working standard solution, manual sample preparation and the four accroma\textsuperscript{TM} robot sample preparations respectively. All chromatograms can be found in the appendix B.
4.2. Working standard

Sample Name: Working Standard

=====================================================================
Acq. Operator : SYSTEM                         Seq. Line :   1
Acq. Instrument : Agilent Demo LC                 Location :   P2-K-01
Injection Date  : 8/28/2018 5:27:43 PM                 Inj :   1
Inj Volume : 50.000 µl
Method          : C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed    : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

=====================================================================

Area Percent Report

Sorted By :      Signal
Multiplier :      1.0000
Dilution :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100
Peak RetTime Type  Width     Area      Height     Area
#   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
1   2.605 BB    0.1040   27.38193    3.89538   0.8492
2   4.010 MM    0.1227 3197.05737  434.13150  99.1508

Totals :                  3224.43930  438.02688

=====================================================================

*** End of Report ***
4.3. Manual sample preparation

Data File C:\Chem32\b20180828_electrolab 2018-08-28 17-26-16\2018-08-28-P2-F1-Manual.D
Sample Name: Manual

Acq. Operator : SYSTEM                         Seq. Line :   7
Acq. Instrument : Agilent Demo LC                 Location :   P2-F-01
Injection Date  : 8/28/2018 6:27:54 PM                 Inj :   1
Inj Volume : 50.000 µl
Method          : C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed    : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

Area Percent Report

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>[min]</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.592</td>
<td>MM</td>
<td>0.0710</td>
<td>5.33519</td>
<td>1.25247</td>
</tr>
<tr>
<td>2</td>
<td>2.658</td>
<td>BB</td>
<td>0.1598</td>
<td>24.58152</td>
<td>2.08153</td>
</tr>
<tr>
<td>3</td>
<td>3.391</td>
<td>BB</td>
<td>0.0914</td>
<td>5.87314</td>
<td>1.01710</td>
</tr>
<tr>
<td>4</td>
<td>4.019</td>
<td>BB</td>
<td>0.1091</td>
<td>3234.10205</td>
<td>454.09500</td>
</tr>
</tbody>
</table>

Totals : 3269.89190 458.44611
4.4. accroma™ sample 1

Data File C:\Chem32\20180828_electrolab 2018-08-28 17-26-16\2018-08-28-P2-F2-accroma 1.D
Sample Name: accroma 1

==================================================================
Acq. Operator : SYSTEM                         Seq. Line : 9
Acq. Instrument : Agilent Demo LC                 Location : P2-F-02
Injection Date : 8/28/2018 6:47:57 PM                 Inj : 1
Inj Volume : 50.000 µl
Method : C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

Area Percent Report
==================================================================

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>[min]</th>
<th>[min]</th>
<th>[mAU]*s</th>
<th>[mAU]</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.549 MM</td>
<td>0.0805</td>
<td>40.60861</td>
<td>8.41215</td>
<td>1.2063</td>
</tr>
<tr>
<td>2</td>
<td>2.652 BB</td>
<td>0.1573</td>
<td>23.88739</td>
<td>2.09065</td>
<td>0.7096</td>
</tr>
<tr>
<td>3</td>
<td>3.389 VB</td>
<td>0.1616</td>
<td>20.74656</td>
<td>1.78489</td>
<td>0.6163</td>
</tr>
<tr>
<td>4</td>
<td>4.018 BB</td>
<td>0.1093</td>
<td>3281.19849</td>
<td>459.54834</td>
<td>97.4679</td>
</tr>
</tbody>
</table>

Totals : 3366.44104 471.83603

==================================================================
4.5. accroma™ sample 2

Data File C:\Chem32\20180828_electrolab 2018-08-28 17-26-16\2018-08-28-P2-F3-accroma 2.D
Sample Name: accroma 2

=====================================================================
Acq. Instrument : Agilent Demo LC                 Location :   P2-F-03
Injection Date  : 8/28/2018 7:28:03 PM                 Inj :   1
Inj Volume : 50.000 µl
Method          : C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed    : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

Area Percent Report
=====================================================================
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
#   [min]        [min]   [mAU*s]     [mAU]        %
---|-------|----|-------|----------|----------|--------|
1   1.552 BV R  0.0771   31.66964    6.25232   0.9350
2   2.654 BB    0.1515   21.45221    1.93334   0.6333
3   3.054 BB    0.0997   11.44653    1.71792   0.3379
4   3.384 BB    0.0915    5.91304    1.02223   0.1746
5   4.018 BB    0.1092 3316.63330  465.04694  97.9191

Totals :                  3387.11473  475.97274

Agilent Demo LC 8/29/2018 7:49:01 AM SYSTEM
4.6. accroma™ sample 3

Data File C:\Chem32\...0180828_electrolab 2018-08-28 17-26-16\2018-08-28-P2-F4-accroma 3.D
Sample Name: accroma 3

=====================================================================
Acq. Operator : SYSTEM                      Seq. Line : 15
Acq. Instrument : Agilent Demo LC            Location : P2-F-04
Injection Date : 8/28/2018 7:48:05 PM        Inj : 1
Inj Volume : 50.000 µl
Method : C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

Peak RetTime Type Width Area Height Area
#  [min]  [min]  [mAU *s]  [mAU]    %
----|-------|----|-------|----------|----------|--------|
1   1.549 MM 0.0943 26.30566 4.64946   0.7690
2   2.653 BB 0.1692 23.15378 1.86067   0.6768
3   3.045 BB 0.1314  9.34745 1.07551   0.2732
4   4.008 BB 0.1151 3362.06226 450.53397 98.2809

Totals : 3420.86915 458.11960

=====================================================================
4.7. accroma™ sample 4

Data File C:\Chem32\...0180830_electrolab 2018-08-30 11-07-03\2018-08-30-P2-P5-accroma 4.D
Sample Name: accroma 4

=====================================================================
Acq. Operator   : SYSTEM                         Seq. Line :   1
Acq. Instrument : Agilent Demo LC                 Location :   P2-F-05
Injection Date  : 8/30/2018 11:08:42 AM                Inj :   1
Inj Volume : 50.000 µl
Method          : C:\Chem32\1\Data\20180828_electrolab\20180830_electrolab 2018-08-30 11-07-03\ELECTROLAB.M (Sequence Method)
Last changed    : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

Area Percent Report

=====================================================================

Sorted By             :      Signal  
Multiplier            :      1.0000  
Dilution              :      1.0000  
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

Peak RetTime Type   Width     Area      Height     Area
#   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
1   1.555 MM    0.0798   33.21033    6.93188   0.9967
2   2.692 BB    0.1601   23.17923    1.92999   0.6956
3   4.100 BB    0.1122 3275.64014  453.62842  98.3077

Totals :                  3332.02970  462.49029

=====================================================================

*** End of Report ***
4.8. accroma™ Log files

The following image 2 shows a part of the log file, which will be created for every accroma™ prepared sample. You can find the complete log files of every tested accroma™ preparation in the appendix C.

Figure 2.: Excerpt of a sample log file

4.9. Comparison

A full comparison of the manual and robotic sample preparations are summarised below in tables 4 and 5. Table 4 shows the time taken for the extraction (Dry milling and wet extraction) comparing the manual method of ultrasonication with the robotic accroma method and table 5 shows the overall time to completely process a single sample from weighing to transferring the HPLC vial into the liquid chromatograph ready for chromatographic analysis - although not undertaken in this experiment but the robot can be fully integrated with HPLC so there is no need for manual intervention.

As shown in table 4 it was possible to complete the extraction of Levothyroxine Sodium tablets rapidly with the accroma™ robot – manual extraction taking 30 minutes compared to just 3 mins by accroma™ robot. Additionally, a greater recovery of drug was achieved when preparing samples by the accroma™ robot. Even when you consider the overall time for processing a single sample from weighing to transferring the vial to the liquid chromatograph preparation by the accroma™ robot was achieved in significantly less time 14 mins for accroma™ sample 4 vs 45 minutes when following the manual procedure.
<table>
<thead>
<tr>
<th>Preparation method</th>
<th>Extraction time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td>30 min</td>
</tr>
<tr>
<td>accroma™ 1</td>
<td>15 min</td>
</tr>
<tr>
<td>accroma™ 2</td>
<td>6 min</td>
</tr>
<tr>
<td>accroma™ 3</td>
<td>5 min</td>
</tr>
<tr>
<td>accroma™ 4</td>
<td>3 min</td>
</tr>
</tbody>
</table>

Table 4.: Extraction time

<table>
<thead>
<tr>
<th>Sample</th>
<th>Preparation Time</th>
<th>ØPeak area</th>
<th>Concentration</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Standard</td>
<td>-</td>
<td>3187.89 mAU*s</td>
<td>0.0047 mg/ml</td>
<td>-</td>
</tr>
<tr>
<td>Manual</td>
<td>45 min</td>
<td>3234.78 mAU*s</td>
<td>0.00477 mg/ml</td>
<td>100 %</td>
</tr>
<tr>
<td>accroma™ 1</td>
<td>24 min</td>
<td>3284.41 mAU*s</td>
<td>0.00484 mg/ml</td>
<td>102 %</td>
</tr>
<tr>
<td>accroma™ 2</td>
<td>19 min</td>
<td>3317.61 mAU*s</td>
<td>0.00489 mg/ml</td>
<td>103 %</td>
</tr>
<tr>
<td>accroma™ 3</td>
<td>16 min</td>
<td>3359.80 mAU*s</td>
<td>0.00495 mg/ml</td>
<td>104 %</td>
</tr>
<tr>
<td>accroma™ 4</td>
<td>14 min</td>
<td>3274.85 mAU*s</td>
<td>0.00483 mg/ml</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Table 5.: Comparison
5. Discussion

Extraction times by the accroma™ robot were shown to be significantly shorter than by the manual method of ultrasonication (3 mins vs 30 mins for accroma™ robot and a manually prepared sample respectively). Not only was full extraction achieved in 2 minutes by the accroma™ robot but in addition a slightly greater recovery was achieved when compared to the manual method. When considering the overall time of preparing a single sample from weighing to transferring the HPLC vial into the liquid chromatograph ready for chromatographic analysis a significant reduction in sample preparation time was observed when preparing sample by the accroma™ robot 14 mins compared to 45 minutes by manual means. When considering multiple samples are usually prepared for uniformity of content (10 tablets are usually taken) and often multiple batches require analysis the accroma™ robot offers a very significant cost saving by significantly reducing sample preparation time. Also as the accroma™ robot is fully integrated to HPLC there is no need for manual intervention once the sample has been placed in the robots extraction tube. Other considerations are the audit data trail that is available from the accroma™ robot and how this increases data integrity and compliance. Throughout this study no degradation of samples or adsorption was observed for any of the samples.
Part II.

Appendix
A. Literature

On the following pages the relevant literature for this report can be found.
Levothyroxine Sodium Tablets

Levothyroxine Sodium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levothyroxine sodium (C₁₅H₁₀I₄NNaO₄).

(Official October 3, 2009)

Change to read:

Levothyroxine Sodium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levothyroxine sodium (C₁₅H₁₀I₄NNaO₄).

(Official October 3, 2009)

Package and storage— Preserve in tight, light-resistant containers. Do not use paddle stirrers with synthetic coating.

USP Reference standards (1) — USP Levothyroxine RS; USP Lithotrycine RS

Change to read:

Levothyroxine Sodium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levothyroxine sodium (C₁₅H₁₀I₄NNaO₄).

(Official October 3, 2009)

Medium, Apparatus, Procedure— Dissolve 0.10 g of USP Levothyroxine RS in 100 mL of water, and dilute with water to 1000 mL. Use this solution as the medium.

Mobile phase— Prepare a stock solution by transferring about 100 mg of USP Levothyroxine RS, accurately weighed, to a 100-mL volumetric flask. Add 50 mL of methanol, mix, dilute with water to 100 mL, and mix. Prepare a mixed solution by transferring about 0.1 mg of USP Levothyroxine RS in methanol having a known concentration of about 5 mg per mL, to a centrifuge tube, and dilute with methanol to about 10 mL. Prepare a standard solution by diluting the stock solution with water to 100 mL.

Apparatus— Chromatograph the standard solution, and record the chromatograms, and measure the responses for the major peaks. Calculate the amount of C₁₅H₁₀I₄NNaO₄ dissolved in 15 minutes, by employing the following method.

1. The amount of C₁₅H₁₀I₄NNaO₄ dissolved is not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levothyroxine sodium (C₁₅H₁₀I₄NNaO₄), as determined by the Assay preparation.

Limit of inorganic substances— Separate and determine the amounts of sodium and potassium in the Assay preparation, and record the results. Calculate the amount of C₁₅H₁₀I₄NNaO₄ dissolved in 45 minutes, by employing the following method.

Limit of tartrazine— Dissolve an amount of the Assay preparation, equivalent to about 100 mg of levothyroxine sodium, in a centrifuge tube, and dilute with methanol to about 10 mL. Transfer to a volumetric flask, add a known amount of tartrazine to the solution, and dilute with methanol to about 10 mL. Prepare an intermediate solution by diluting the stock solution with methanol to about 0.1 mg per mL, and make adjustments if necessary to suit the sensitivity of the procedure.

System suitability— Prepare a solution of USP Levothyroxine RS in methanol having a known concentration of about 5 mg per mL. Dilute this stock solution with methanol to obtain a solution having a concentration similar to that expected in the Test solution. Prepare a solution of USP Levothyroxine RS in methanol having a known concentration of about 5 mg per mL. Dilute this stock solution with methanol to obtain a solution having a concentration similar to that expected in the Test solution.

Chromatograms— Record the chromatograms, and measure the responses for the major peaks. Calculate the amount of C₁₅H₁₀I₄NNaO₄ dissolved in 45 minutes, by employing the following method.

Limit of related substances— The chromatograms obtained in the Assay preparation and in the Standard solution show no peak that is more intense than the peak corresponding to tartrazine in the Standard solution.

Uniformity of dosage units (935)— Meet the requirements.

Summary— The Assay preparation contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levothyroxine sodium (C₁₅H₁₀I₄NNaO₄), as determined by the Assay preparation.
B. Chromatograms

On the following pages all chromatograms can be found.
B.1. Chromatograms of the working standard

Sample Name: Working Standard

=================================================================================================
Acq. Operator : SYSTEM                         Seq. Line : 1
Acq. Instrument : Agilent Demo LC                 Location : P2-E-01
Injection Date  : 8/28/2018 5:27:43 PM                Inj : 1
Inj Volume : 50.000 µl
Method          : C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed    : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

Area Percent Report
=================================================================================================
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>[min]</th>
<th>[min]</th>
<th>[mAU*s]</th>
<th>[mAU]</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.605</td>
<td>BB</td>
<td>0.1040</td>
<td>27.38193</td>
<td>3.89538</td>
</tr>
<tr>
<td>2</td>
<td>4.010</td>
<td>MM</td>
<td>0.1227</td>
<td>3197.05737</td>
<td>434.13150</td>
</tr>
</tbody>
</table>

Totals : 3224.43930 438.02688

=================================================================================================

*** End of Report ***

Agilent Demo LC 8/29/2018 7:46:53 AM SYSTEM
Page 1 of 1
### Area Percent Report

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000
Use Multiplier & Dilution Factor with ISTDs

#### Signal 1: DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>RetTime [min]</th>
<th>Width [min]</th>
<th>Area [mAU*s]</th>
<th>Height [mAU]</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.601</td>
<td>0.1066</td>
<td>29.00041</td>
<td>4.00150</td>
<td>0.9014</td>
</tr>
<tr>
<td>2</td>
<td>4.001</td>
<td>0.1112</td>
<td>3188.43921</td>
<td>436.49219</td>
<td>99.0986</td>
</tr>
</tbody>
</table>

Totals: 3217.43962 440.49368

---

### Additional Information

- Peak(s) manually integrated

---

## Data File


Sample Name: Working Standard
Acq. Operator: SYSTEM  Seq. Line: 3
Acq. Instrument: Agilent Demo LC  Location: P2-E-01
Injection Date: 8/28/2018 5:47:46 PM  Inj: 3
Inj Volume: 50.000 µl
Method: C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed: 8/16/2018 4:28:44 PM by SYSTEM
Additional Info: Peak(s) manually integrated

---

Area Percent Report

---

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>[min]</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.601</td>
<td>BB</td>
<td>0.1048</td>
<td>29.09075</td>
<td>4.0061</td>
</tr>
<tr>
<td>2</td>
<td>4.002</td>
<td>BB</td>
<td>0.1132</td>
<td>3184.99365</td>
<td>435.93277</td>
</tr>
</tbody>
</table>

Totals: 3214.08440 439.93338

---

*** End of Report ***
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>[min]</th>
<th>[min]</th>
<th>[mAU*s]</th>
<th>[mAU]</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.601</td>
<td>BB</td>
<td>0.1166</td>
<td>32.98740</td>
<td>4.06906</td>
</tr>
<tr>
<td>2</td>
<td>4.002</td>
<td>BB</td>
<td>0.1110</td>
<td>3182.30029</td>
<td>436.95288</td>
</tr>
</tbody>
</table>

Totals : 3215.28769 441.02194

*** End of Report ***
**Area Percent Report**

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>Peak RetTime Type Width [min]</th>
<th>Area [mAU*s]</th>
<th>Height [mAU]</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2.601 BB</td>
<td>0.1050</td>
<td>27.99032</td>
<td>0.8710</td>
</tr>
<tr>
<td>2 4.001 BB</td>
<td>0.1112</td>
<td>3185.62183</td>
<td>99.1290</td>
</tr>
</tbody>
</table>

Totals: 3213.61215 440.13900

*** End of Report ***
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>[min]</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.602 BB</td>
<td>0.1049</td>
<td>28.17949</td>
<td>3.96620</td>
<td>0.8765</td>
</tr>
<tr>
<td>2</td>
<td>4.003 BB</td>
<td>0.1110</td>
<td>3186.70996</td>
<td>437.13452</td>
<td>99.1235</td>
</tr>
</tbody>
</table>

Totals : 3214.88946 441.10072

*** End of Report ***
Acq. Operator : SYSTEM                         Seq. Line :  11
Acq. Instrument : Agilent Demo LC                 Location :   P2-E-01
Injection Date  : 8/28/2018 7:08:00 PM                 Inj :   1
Inj Volume : 50.000 µl
Method          : C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed    : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

Area Percent Report

Sorted By               :      Signal
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

#     [min]     [min]     [mAU*s]  [mAU]     [%]
----|-------|----|-------|----------|----------|--------|
1    2.600 BB  0.1022  28.18826  4.00108   0.8767
2    4.002 BB  0.1126  3187.06079 439.40607  99.1233

Totals :                  3215.24905  443.40715

*** End of Report ***
Area Percent Report

Sorted By: Signal  
Multiplier: 1.0000  
Dilution: 1.0000  
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

| Peak RetTime Type Width Area Height Area % |
|------|-------|-----|-------|-----|
| 1    | 2.601 | BB  | 0.1035 | 28.12677 | 4.02810 | 0.8754 |
| 2    | 4.002 | BB  | 0.1105 | 3184.87842 | 439.63834 | 99.1246 |

Totals: 3213.00518 443.66644

*** End of Report ***
Acq. Operator : SYSTEM                         Seq. Line :  17
Acq. Instrument : Agilent Demo LC                 Location :   P2-E-01
Injection Date  : 8/28/2018 8:08:06 PM                 Inj :   1
Inj Volume : 50.000 µl
Method          : C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-
                16\ELECTROLAB.M (Sequence Method)
Last changed    : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225.4 Ref=360.100

Peak RetTime Type  Width     Area      Height     Area
#    [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
1   2.602 BB    0.1021   27.52135    4.00763   0.8559
2   4.003 BB    0.1104 3187.95776  440.58823  99.1441
Totals :                  3215.47911  444.59586

*** End of Report ***
Sample Name: Working Standard

=====================================================================
Acq. Operator   : SYSTEM                         Seq. Line : 18
Acq. Instrument : Agilent Demo LC                 Location : P2-K-01
Injection Date  : 8/28/2018 8:18:07 PM                 Inj : 2
Inj Volume : 50.000 µl
Method          : C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed    : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>RetTime Type</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1106 BB</td>
<td>32.10922</td>
<td>4.13425</td>
<td>0.9953</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.1105 BB</td>
<td>3193.89575</td>
<td>441.01691</td>
<td>99.0047</td>
<td></td>
</tr>
</tbody>
</table>

Totals : 3226.00497 445.15116

*** End of Report ***

Agilent Demo LC 8/29/2018 7:49:53 AM SYSTEM  Page 1 of 1
Acq. Operator   : SYSTEM                         Seq. Line :   8
Acq. Instrument : Agilent Demo LC                 Location :   P2-F-01
Injection Date  : 8/28/2018 6:37:56 PM                 Inj :   2
Inj Volume : 50.000 µl
Method          : C:\Chem32\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed    : 8/16/2018 4:28:44 PM by SYSTEM
Additional Info : Peak(s) manually integrated

Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>RetTime</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[min]</td>
<td>[min]</td>
<td>[mAU*s]</td>
<td>[mAU]</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.589</td>
<td>0.0788</td>
<td>6.49078</td>
<td>1.37212</td>
<td>0.1987</td>
</tr>
<tr>
<td>2</td>
<td>2.655</td>
<td>0.1606</td>
<td>24.98134</td>
<td>2.10310</td>
<td>0.7647</td>
</tr>
<tr>
<td>3</td>
<td>4.018</td>
<td>0.1091</td>
<td>3235.45483</td>
<td>454.11005</td>
<td>99.0366</td>
</tr>
</tbody>
</table>

Totals :                  3266.92695  457.58527

*** End of Report ***
### Area Percent Report

<table>
<thead>
<tr>
<th>Signal</th>
<th>RetTime</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal</td>
<td>1.549</td>
<td>0.0805</td>
<td>40.60861</td>
<td>8.41215</td>
<td>1.2063</td>
</tr>
<tr>
<td>Signal</td>
<td>2.652</td>
<td>0.1573</td>
<td>23.88739</td>
<td>2.09065</td>
<td>0.7096</td>
</tr>
<tr>
<td>Signal</td>
<td>3.389</td>
<td>0.1616</td>
<td>20.74656</td>
<td>1.78489</td>
<td>0.6163</td>
</tr>
<tr>
<td>Signal</td>
<td>4.018</td>
<td>0.1033</td>
<td>3281.19849</td>
<td>459.54034</td>
<td>97.4679</td>
</tr>
</tbody>
</table>

**Totals:** 3366.44104 471.83603

---

**B.3. Chromatograms of the accroma™1 sample preparation**
Acq. Operator: SYSTEM                         Seq. Line: 10
Acq. Instrument: Agilent Demo LC                 Location: P2-F-02
Injection Date: 8/28/2018 6:57:58 PM                 Inj: 2
Inj Volume: 50.000 µl
Method: C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed: 8/16/2018 4:28:44 PM by SYSTEM
Additional Info: Peak(s) manually integrated

Area Percent Report
Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100 Use Multiplier & Dilution Factor with ISTDs

<table>
<thead>
<tr>
<th>#</th>
<th>RetTime</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.549</td>
<td>0.0819</td>
<td>41.48192</td>
<td>8.44478</td>
<td>1.2293</td>
</tr>
<tr>
<td>2</td>
<td>2.653</td>
<td>0.1554</td>
<td>23.83518</td>
<td>2.11661</td>
<td>0.7064</td>
</tr>
<tr>
<td>3</td>
<td>3.385</td>
<td>0.1626</td>
<td>21.47165</td>
<td>1.83415</td>
<td>0.6363</td>
</tr>
<tr>
<td>4</td>
<td>4.016</td>
<td>0.1094</td>
<td>3287.62378</td>
<td>460.13019</td>
<td>97.4280</td>
</tr>
</tbody>
</table>

Totals: 3374.41253 472.52573
B.4. Chromatograms of the accroma™ 2 sample preparation

---

### Area Percent Report

<table>
<thead>
<tr>
<th>Peak Retime Type</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>[min]</td>
<td>[min]</td>
<td>[mAU*s]</td>
<td>[mAU]</td>
</tr>
<tr>
<td>----</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>1</td>
<td>1.552</td>
<td>BV</td>
<td>0.0771</td>
<td>31.66964</td>
</tr>
<tr>
<td>2</td>
<td>2.654</td>
<td>BB</td>
<td>0.1515</td>
<td>21.45221</td>
</tr>
<tr>
<td>3</td>
<td>3.384</td>
<td>BB</td>
<td>0.0915</td>
<td>5.91304</td>
</tr>
<tr>
<td>5</td>
<td>4.018</td>
<td>BB</td>
<td>0.1092</td>
<td>3316.63330</td>
</tr>
</tbody>
</table>

**Totals:** 3387.11473 475.97274

---

Additional Info: Peak(s) manually integrated
### Area Percent Report

**Sorted By:**  | Signal  
**Multiplier:**  | 1.0000  
**Dilution:**  | 1.0000  

Use Multiplier & Dilution Factor with ISTDs

**Signal:** DAD1 C, Sig=225,4 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>RetTime Type</th>
<th>Width [min]</th>
<th>Area [mAU*s]</th>
<th>Height [mAU]</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BB</td>
<td>0.0752</td>
<td>29.33980</td>
<td>6.20330</td>
<td>0.8647</td>
</tr>
<tr>
<td>2</td>
<td>BV</td>
<td>0.1677</td>
<td>25.55798</td>
<td>2.04709</td>
<td>0.7533</td>
</tr>
<tr>
<td>3</td>
<td>VV</td>
<td>0.1059</td>
<td>13.19306</td>
<td>1.83526</td>
<td>0.3888</td>
</tr>
<tr>
<td>4</td>
<td>VB</td>
<td>0.0929</td>
<td>6.25426</td>
<td>1.05963</td>
<td>0.1843</td>
</tr>
<tr>
<td>5</td>
<td>BB</td>
<td>0.1091</td>
<td>3318.59131</td>
<td>465.99088</td>
<td>97.8088</td>
</tr>
</tbody>
</table>

**Totals:** 3392.93641 477.13616

---

**Additional Info:** Peak(s) manually integrated
B.5. Chromatograms of the accroma™ 3 sample preparation

Area Percent Report

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225.4 Ref=360.100
Peak RetTime Type  Width     Area      Height     Area
#   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
1   1.549 MM    0.0943   26.30566    4.64946   0.7690
2   2.653 BB    0.1692   23.15378    1.86067   0.6768
3   3.045 BB    0.1314    9.34745    1.07551   0.2732
4   4.008 BB    0.1151 3362.06226  450.53397  98.2809

Totals :                  3420.86915  458.11960

Area Percent Report

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225.4 Ref=360.100
Peak RetTime Type  Width     Area      Height     Area
#   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
1   1.549 MM    0.0943   26.30566    4.64946   0.7690
2   2.653 BB    0.1692   23.15378    1.86067   0.6768
3   3.045 BB    0.1314    9.34745    1.07551   0.2732
4   4.008 BB    0.1151 3362.06226  450.53397  98.2809

Totals :                  3420.86915  458.11960
Acq. Operator: SYSTEM
Seq. Line: 16
Acq. Instrument: Agilent Demo LC
Location: P2-F-04
Injection Date: 8/28/2018 7:58:05 PM
Inj: 2
Inj Volume: 50.000 µl
Method: C:\Chem32\1\Data\20180828_electrolab\20180828_electrolab 2018-08-28 17-26-16\ELECTROLAB.M (Sequence Method)
Last changed: 8/16/2018 4:28:44 PM by SYSTEM
Additional Info: Peak(s) manually integrated

Area Percent Report

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000
Use Multiplier & Dilution Factor with ISTDs
Signal 1: DAD1 C, Sig=225,4 Ref=360,100

Peak RetTime Type  Width  Area      Height     Area       %
#   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|-----|-------|----------|----------|--------|
1   1.549 MM    0.1008  31.56010    5.21596   0.9224
2   2.654 BB    0.1635  23.07664    1.90305   0.6744
3   3.045 BB    0.1260  9.12931     1.08629   0.2668
4   4.010 BB    0.1146 3357.79785  452.27109  98.1363
Totals:                  3421.56390  460.47639
B.6. Chromatograms of the accroma™ 4 sample preparation

Data File C:\Chem32\...0180830_electrolab 2018-08-30 11-07-03\2018-08-30-P2-F5-accroma 4.D
Sample Name: accroma 4

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=225,4 Ref=360,100

Peak RetTime Type Width Area Height Area
# [min] [min] [mAU*s] [mAU] %
--- ----- ----- --------- ------ ------
1 1.555 MM 0.0798 33.21033 6.93188 0.9967
2 2.692 BB 0.1601 23.17923 1.92999 0.6956
3 4.100 BB 0.1122 3275.64014 453.62842 98.3077

Totals : 3332.02970 462.49029

*** End of Report ***
C. Sample log files

On the following pages the sample log files of every accroma™ prepared sample can be found.
1. Shaking

2. Preparation of consumables

3. Aspiration sample

4. Cooling

5. Aspiration sample to liquid module

6. Transfer of snapvial bottom to liquid module

7. Transfer of snapvial top to liquid module

8. Aspiration of filtered sample

9. Replacement of snapvial

10. Replacement of extraction tube

11. Temperature balance

12. Workflow completed
2018-08-28 16:08:07.999 action=shaking start
2018-08-28 16:08:07.999 shaker_active=yes
2018-08-28 16:08:08.089 preparation_consumables=yes
2018-08-28 16:08:08.089 LM_three=1
2018-08-28 16:08:08.416 aspiration_sample=excrimp20180828140150
2018-08-28 16:13:15.459 action=shaking end
2018-08-28 16:13:15.459 cooldown2=0
2018-08-28 16:13:15.459 shaker_active=no
2018-08-28 16:13:15.544 device4=shaker
2018-08-28 16:13:15.630 device5=shaker
2018-08-28 16:13:15.794 device6=shaker
2018-08-28 16:13:15.880 LM_three=1
2018-08-28 16:13:15.880 needle_three=1
2018-08-28 16:13:15.991 detachwait=Z1
2018-08-28 16:13:46.695 action=transfer sample to liquidmodule
2018-08-28 16:13:46.959 action=transfer sample to liquidmodule
2018-08-28 16:13:47.047 tip_filter=2
2018-08-28 16:13:47.136 filter_id2=filter20180828160250
2018-08-28 16:13:47.301 action=transfer snapvial bottom to liquidmodule
2018-08-28 16:13:47.389 aspiration_sample=excrimp20180828140150
2018-08-28 16:13:47.389 preparation_consumables=yes
2018-08-28 16:13:47.473 action=transfer snapvial top to liquidmodule
2018-08-28 16:13:47.569 sample_original=excrimp20180828140150
2018-08-28 16:13:47.569 sample_filtered=snapvial20180828140150
2018-08-28 16:13:47.569 snapvial_slot=SnapvialslotB5
2018-08-28 16:13:50.254 cooldown5=0
2018-08-28 16:14:30.799 needle_slot3=Needleslot2
2018-08-28 16:14:30.799 needle_last=2
2018-08-28 16:14:30.799 extractionspeed2=144.000000
2018-08-28 16:15:44.372 action=conditioning filter
2018-08-28 16:16:01.343 snapdispvol=1530
2018-08-28 16:16:13.678 action=dispense through filter
2018-08-28 16:17:32.721 action=transfer snapvialtop to snapvial bottom
2018-08-28 16:17:40.459 sample_original=excrimp20180828140150
2018-08-28 16:17:40.459 sample_filtered=snapvial20180828140150
2018-08-28 16:17:40.459 snapvial_slot=SnapvialslotB5
2018-08-28 16:18:13.816 preparation_consumables=no
2018-08-28 16:18:13.816 filterSetRes=null
2018-08-28 16:18:13.816 action=replace snapvial
2018-08-28 16:18:13.816 device10=null
2018-08-28 16:18:47.941 action=replace extractiontube
2018-08-28 16:18:48.101 PumpCorrectionFactor1=1.000000
2018-08-28 16:18:48.101 PumpCorrectionDate1=1904-01-01T01:33:38
2018-08-28 16:18:48.101 PumpCorrectionFactor2=1.000000
2018-08-28 16:18:48.101 PumpCorrectionDate2=1904-01-01T01:33:38
2018-08-28 16:18:48.115 WorkflowStep 7 completed sampleprep method 20180828_Robo2_electrolab
2018-08-28 16:18:50.159 WorkflowStep 8 completed End Workflow
2018-08-28 16:18:50.159 Workflow completed