

# Are TripleTOF<sup>®</sup> systems the future for HT-ADME/toxicity screening?



### Agenda

#### **Overview of Cyprotex**

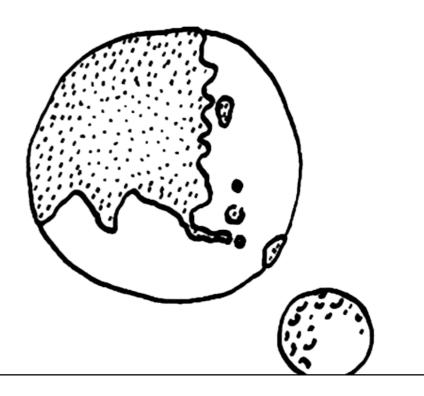
**Overview of HT Processes** 

LC-MS Maintenance/Daily checks

Overview of MS data handling

Soft spot Met ID analysis

**Future Plans** 





### Cyprotex

Specialists in in vitro and in silico ADME-Tox Services

- Established in 1999, Cyprotex Limited was acquired by Evotec AG in 2016
- As part of the Evotec group, Cyprotex focuses on the ADME-Tox
- Our clients mainly focus on pharmaceutical research, however, we also support clients in the chemical, cosmetics and personal care, agrichemical and tobacco industries
- We specialise in *in vitro* ADME-Tox services offering screening assays within drug discovery projects, this can then be followed by regulatory *in vitro* ADME and DDI studies in preclinical and clinical development
- To perform tight turnaround deadlines with high quality data our scientists are supported by a team of software engineers who have developed an in-house LIMS system (Labsys<sup>™</sup>) that is constantly developing to fulfil the ever changing needs of our science and client requests
- Our scientists are separated out into dedicated specialised teams Permeability/Transporters, Drug Metabolism, Physical Chemistry and Toxicology much of which is supported by a central Analytical hub with a dedicated team offering expertise in LC-MS analysis



### **Cyprotex (continued)**

- LC-MS or LC-MS/MS provides the analytical endpoint for the majority of the discovery assays
- To fit client demand we currently run 18 TQ and 6 HRAM instruments (including 5 SCIEX TripleTOF 6600 and 6600+ LC-MS/MS systems) that acquire data up to 24 hours a day
- Client expectations continue to move towards shorter turn around times while still keeping high quality data. Compound
  optimisation processes (even with an automated systems) still takes a minimum of an overnight run to achieve using TQ
  workflows whereas using a HRAM removes this need therefore compounds can be analysed the day they arrive.
- On average Cyprotex analyse approx. 75,000 compounds a year



### **Cyprotex Process**

#### Laboratory Workflow 2019

| 73,219<br>compounds             | 5,864<br>plates made up     | 8,249<br>assays                     | 5,804,813 samples measured | 377,789<br>results generated |
|---------------------------------|-----------------------------|-------------------------------------|----------------------------|------------------------------|
|                                 |                             |                                     |                            |                              |
| Compound<br>receipt             | Assay plate preparation     | Experimental set up and performance | Sample<br>measurement      | Result processing and QC     |
|                                 |                             | fredom tra                          | Service Services           |                              |
|                                 |                             |                                     |                            |                              |
| 11,109<br>test compound vessels | 5,864<br>assay input plates | 28,433<br>analysis plates           | 1,146,776<br>MS injections | 4,431,234<br>peak areas      |



## SCIEX TripleTOF 6600 and 6600+ LC-MS/MS Systems

Instrument set-up





### Triple Quadrupoles MS/MS vs TTOF MS

Which has the most advantages?

| Triple Quadrupoles   | TTOF  |
|--|---|
| Highly selective optimisation – also possible on the TTOF if run MS/MS | Samples run same day as compound arrival  |
| Smaller foot print   | Ability to boyo lorge consette cizes  |
| Decreased data size  | Ability to have large cassette sizes  |
| No extra samples run due to CDS which increases run time               | Ability to re-extract data looking for adducts / alternatives masses / metabolites at anytime |
| Instant chromatographic data available                                 | Addition of different IS then extract data using the one you want                             |



### **Sample Analysis Workflow**

Once compounds have arrived and registered

| MRM Workflow   |         | TripleTOF®  |
|--|---------|---|
| Compounds added to an optimisation task                  | Day 1   | Compounds run in assay  |
| Compounds optimised in positive and negative mode        | Day 1   | Assay run on instrument in positive ionisation                        |
| Spectra and chromatography processed, if passes in-house | Day 2   | Data extracted on positive ion data, suitable data reported to client |
| criteria optimisation is copied in master database       | Day 2   | If no peak is detected then assay is injected in negative mode        |
| Compounds run in assay                                   | Day 3   | Data extracted on negative ion and results reported to the client     |
| Data processed and reported to client                    | Day 4/5 |   |



### **Current TripleTOF Usage at Cyprotex**

Just the start of all the possibilities

Compatible with all Cyprotex assays but primarily used for clearance (CL) and Chrom Log D (CHI) assays due to compound numbers and turnaround time. Balance between gradient run time required to fit in HT demands but also data quality (number of points across the chromatographic peak to quantitation) with the ability to mine for metabolites. Bioanalysis / Toxicology – TOF MS or MRM mode (sensitivity mode). For poorly fragmenting or very small molecules in TOF MS. Ability to extract on different fragment ions without having to re-inject, also to be able to optimise compounds and run MS/MS if a more specific transition is required.



### **Maintenance and Performance Checks**

Performed on a weekly/daily basis

- Weekly clean of the curtain plate
- Performance checks run in positive (5 known compounds) and negative (2 known compounds). Evaluation of linearity, sensitivity and reproducibility in TOF MS. Identification of possible instrument issues including sensitivity, peak shape, retention time shift etc. Negative run focuses on negative sensitivity for MS detection.
- MS and MS/MS mass accuracy is checked daily on 3 compounds
- System "optimisation" run when sensitivity drops on instrument and manual infusions indicates the instrument has lost accuracy. If system optimisation is performed too frequently the MS/MS accuracy may be compromised.
- Deep clean (QJet Ion Guide and orifice plate) performed when sensitivity drops below 50% of average peak area response. Vent and pump down usually takes 12 hours. Spare clean QJet ion guides reduces this time to <6 hours.
- CDS-calibration delivery system is run at the start of every batch and then run once an hour



### **Performance Check**

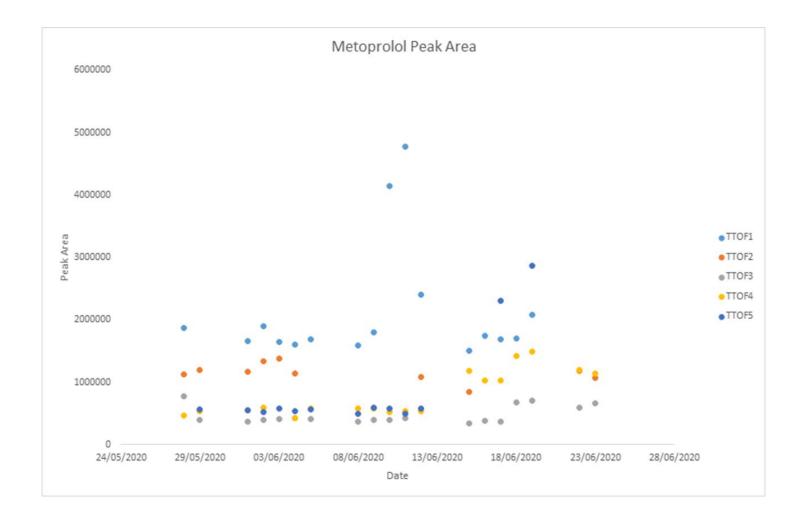
#### MultiQuant<sup>™</sup> Software Data

| ndex   | Sample Name  | Sample ID  | -  | 🗱 🔍 📑 📄   | IS | IS Name                    | Component            | Outier        | Actual               | Area                          | Height                               | Retention                      | Signal / Noise                             | Ilead    | Calculated                | Accuracy          | Width at 5%     | Taing          | *BatchDat_  | *Cor |
|--|--|--|--|---|----|----------------------------|----------------------|---------------|----------------------|-------------------------------|--------------------------------------|--------------------------------|--|----------|---------------------------|-------------------|-----------------|----------------|-------------|------|
| 1  | Wash   | Checks 270520  | Solvent                                    | 27/05/2020 08:06:39   |    | (No IS)                    | Group Name           | Reasons       | Concentration<br>N/A | 2.751e3                       | 6.655e2                              | Time<br>0.83                   | 18.2                                       |          | Concentration<br>7.082e-1 | N/A               |                 | Factor<br>0.55 | Job 8700    |      |
| 7  | s0   | Checks_270520  | Standard                                   | 27/05/2020 08:09:17   |    | (No IS)                    | a                    | Accuracy      | 0.00                 | 6.282e1                       | 1.017e2                              | 0.85                           | 7.3  | <b>V</b> | 3.601e-1                  | N/A               |                 | 1.36           | Job_8700    | -    |
| 13   | s1   | Checks 270520  | Standard                                   | 27/05/2020 08:03:17   |    | (No IS)                    | a                    | Accuracy      | 1.00                 | 5.003e3                       | 2.987e3                              | 0.80                           | 248.3                                      | 7        | 1.000e0                   | 100.00            |                 | 1.83           | Job 8700_   | -    |
| 19   | \$2  | Checks_270520  | Standard                                   | 27/05/2020 08:14:29   |    | (No IS)                    | a                    | -             | 10.00                | 7.408e4                       | 3.226e4                              | 0.80                           | 3025.1                                     | V        | 1.000e0                   | 100.00            |                 | 0.84           | Job_8700_   | -    |
| 25   | \$3  | Checks_270520  | Standard                                   | 27/05/2020 08:17:06   |    | (No IS)                    | a                    |               | 100.00               | 6.268e5                       | 3.045e5                              | 0.80                           | 23175.1                                    |          | 8.605e1                   | 86.05             |                 | 1.00           | Job_8700_   | -    |
| 31   | s4   | Checks_270520  | Standard                                   | 27/05/2020 08:19:44   |    | (No IS)                    | a                    |               | 500.00               | 2.665e6                       | 1.393e6                              | 0.81                           | 74936.1                                    |          | 5.000e2                   | 100.00            |                 | 1.13           | Job_8700    | -    |
| 37   | Wash   | Checks_270520  | Solvent                                    | 27/05/2020 08:22:22   |    | (No IS)                    | a                    |               | N/A                  | 1.114e2                       | 1.128e2                              | 0.82                           | 12.9                                       | ~        | 3.664e-1                  | N/A               |                 | 0.81           | Job_8700_   | -    |
| 43   | Wash   | Checks_270520  | Solvent                                    | 27/05/2020 08:25:00   |    | (No IS)                    | a                    |               | N/A                  | 4.952e1                       | 1.026e2                              | 0.89                           | 12.4                                       | v        | 3.584e-1                  | N/A               |                 | 1.85           | Job_8700_   | -    |
| 49   | Wash   | Checks_270520  | Solvent                                    | 27/05/2020 08:27:37   |    | (No IS)                    | a                    |               | N/A                  | 1.650e2                       | 9.449e1                              | 0.90                           | 12.2                                       | <b>v</b> | 3.733e-1                  | N/A               |                 | 2.09           | Job_8700_   | -    |
| 55   | QCS 100 Dil/1  | Checks 270520  |  | 27/05/2020 08:30:14   |    | (No IS)                    | a                    |               | 100.00               | 7.257e5                       | 3.599e5                              | 0.80                           | 33240.5                                    | <b>v</b> | 1.005e2                   | 100.51            |                 | 0.98           | Job 8700_   | -    |
| 61   | QCS 100 Dil/1  | Checks 270520  |  | 27/05/2020 08:32:50   |    | (No IS)                    | a                    |               | 100.00               | 7.216e5                       | 3.939e5                              | 0.80                           | 32353.8                                    | v<br>V   | 9.991e1                   | 99.91             |                 | 1.09           | Job_8700_   | -    |
| 67   | QCS 100 Dil/1  | Checks 270520  |  | 27/05/2020 08:35:27   |    | (No IS)                    | a                    |               | 100.00               | 7.571e5                       | 3.610e5                              | 0.80                           | 32394.2                                    | v<br>V   | 1.052e2                   | 105.17            |                 | 1.22           | Job 8700_   | -    |
| 73   | QCS 100 Dil/1  | Checks_270520  |  | 27/05/2020 08:38:03   |    | (No IS)                    | a                    |               | 100.00               | 7.944e5                       | 4.189e5                              | 0.80                           | 37411.8                                    | <b>v</b> | 1.108e2                   | 110.75            |                 | 1.01           | Job_8700_   | -    |
| 79   | QCS 100 Dil/1  | Checks_270520  |  | 27/05/2020 08:40:40   |    | (No IS)                    | a                    | -             | 100.00               | 6.455e5                       | 3.924e5                              | 0.80                           | 34437.9                                    | v<br>V   | 8.876e1                   | 88.76             |                 | 1.07           | Job_8700_   | -    |
|  | QC3100 DII/1   | CII60K5_270020   | Quality Consol                             | 2//03/2020 00.40.40   |    | (14013)                    | a                    |               | 100.00               | 0.40060                       | 3.32463                              | 0.00                           | 34437.3                                    |          | 0.07061                   | 00.70             | 0.000           | 1.07           | 000_0/00    |      |
| ▶ Ę  | 🗜 🕀 🕅 🛍 🔍 🚍 🚍<br>Apply   | (10: QCS 100 DI/1  | Manual Integra                             | 2001  |    |                            |                      |               |                      | <ul> <li>11: QCS 1</li> </ul> | 00 Dil/1                             |                                |  |          |                           |                   |                 |                |             |      |
| ted F  |  | Area: 7.257e5, Heig  | /000000016 (Quali<br>pht: 3.599e5, RT: 0.8 | ity Control) 267.1667 - 267.16<br>80 min                      |    | :\Analyst Data\Proje<br>80 | cts\System Checks 20 | 019 Q2\Data\C | Checks_270520        | Area: 7.21                    | 6e5, Height: 3                       | 000016 (Qual<br>.939e5, RT: 0. | ity Control) 267.1<br>80 min               | 967 - 26 | 7.1677 - D:\Analyst       | t Data\Projects\! | System Checks 2 | 2019 Q2\Data   | Checks_2705 | 20.  |
| tted F<br>If Win<br>te Exp<br>eport<br>Peak F<br>Peak F  | RT: 0.77 min   | Area: 7.257e5, Heig<br>3e5 -<br>3e5 -<br>1e5 -<br>1e5 -<br>0e0 - | )ht: 3.599e5, RT: 0.8                      | ity Control) 267.1667 - 267.16<br>30 min<br>30 0.4 0.5 0.6 0. | 0  | 80                         | _                    | 019 Q2\Data\C | _                    | Area: 7.21                    | 16e5, Height: 3<br>3e5<br>2e5<br>1e5 | .939e5, RT: 0.                 | ity Control) 267.11<br>30 min<br>3 0.4 0.5 |          |                           | 9 1.0 1.1         |                 |                |             |      |
| ted F<br>It Win<br>te Exp<br>port<br>Peak P<br>egratic   | XT: 0.77 min<br>dow: 10.0 sec<br>pected RT: No ¥<br>Largest Peak<br>Width: 3 point<br>desight: 0.00<br>n Parameters<br>Regression 1 0 4 5  | Area: 7.257e5, Heig<br>3e5<br>2e5<br>1e5<br>0e0                  | o.1 0.2 0.3                                | 90 min<br>1 0.4 0.5 0.6 0.                                    | 0  | 80                         | _                    |               | _                    | Area: 7.21                    | 16e5, Height: 3<br>3e5<br>2e5<br>1e5 | .939e5, RT: 0.                 | 30 min                                     |          | 0.7 0.8 0.5               | 9 1.0 1.1         |                 |                |             |      |
| ted F<br>transformer<br>port<br>Peak N<br>Peak N<br>gration  | RT:         0.77         min           idow:         10.0         sec           pected RT:         No         V           Largest Peak         Width:         3         poin           Height:         0.00         O         O           Parameters | Area: 7.257e5, Heig<br>3e5<br>2e5<br>1e5<br>0e0                  | o.1 0.2 0.3                                | 90 min<br>1 0.4 0.5 0.6 0.                                    | 0  | 80                         | _                    |               | _                    | Area: 7.21                    | 16e5, Height: 3<br>3e5<br>2e5<br>1e5 | .939e5, RT: 0.                 | 30 min                                     |          | 0.7 0.8 0.5               | 9 1.0 1.1         |                 |                |             |      |
| te Exp<br>port<br>Peak V<br>Peak I<br>gratic   | RT:         0.77         min           idow:         10.0         sec           pected RT:         No         V           Largest Peak         Width:         3         poin           Height:         0.00         O         O           Parameters | Area: 7.257e5, Heig<br>3e5<br>2e5<br>1e5<br>0e0                  | o.1 0.2 0.3                                | 90 min<br>1 0.4 0.5 0.6 0.                                    | 0  | 80                         | _                    |               | _                    | Area: 7.21                    | 16e5, Height: 3<br>3e5<br>2e5<br>1e5 | .939e5, RT: 0.                 | 30 min                                     |          | 0.7 0.8 0.5               | 9 1.0 1.1         |                 |                |             |      |
| ted F<br>transformer<br>port<br>Peak N<br>Peak N<br>gration  | XT:         0.77         min           idow:         10.0         sec           pected RT:         No         Y           Largest Peak         Moth:         3           Mdth:         3         poin           Parameters                           | Area: 7.257e5, Heig<br>3e5<br>2e5<br>1e5<br>0e0                  | o.1 0.2 0.3                                | 90 min<br>1 0.4 0.5 0.6 0.                                    | 0  | 80                         | _                    |               | _                    | Area: 7.21                    | 16e5, Height: 3<br>3e5<br>2e5<br>1e5 | .939e5, RT: 0.                 | 30 min                                     |          | 0.7 0.8 0.5               | 9 1.0 1.1         |                 |                |             |      |
| tted F<br>If Win<br>te Exp<br>Peak N<br>Peak N<br>Pe | RT:     0.77     min       idow:     10.0     sec       pected RT:     No     v       Largest Peak     Midth:     3       Width:     3     poin       Height:     0.00     on       Parameters   | Area: 7.257e5, Heig<br>3e5<br>2e5<br>1e5<br>0e0                  | o.1 0.2 0.3                                | 90 min<br>1 0.4 0.5 0.6 0.                                    | 0  | 80                         | _                    |               | _                    | Area: 7.21                    | 16e5, Height: 3<br>3e5<br>2e5<br>1e5 | .939e5, RT: 0.                 | 30 min                                     |          | 0.7 0.8 0.5               | 9 1.0 1.1         |                 |                |             |      |
| cted F<br>alf Win<br>te Exp<br>eport<br>Peak N<br>egratic<br>egration<br>2.56<br>2.00  | XT: 0.77 min<br>dow: 10.0 sec<br>pected RT: No ▼<br>Largest Peak<br>Mdth: 3 poin<br>Height: 0.00<br>on Parameters<br>FRegression 1 1 0 0 0 0<br>Frequencies<br>a6 a<br>a6 a<br>a6 a  | Area: 7.257e5, Heig<br>3e5<br>2e5<br>1e5<br>0e0                  | o.1 0.2 0.3                                | 90 min  | 0  | 80                         | _                    |               | _                    | Area: 7.21                    | 16e5, Height: 3<br>3e5<br>2e5<br>1e5 | .939e5, RT: 0.                 | 30 min                                     |          | 0.7 0.8 0.5               | 9 1.0 1.1         |                 |                |             |      |
| cted F<br>alf Win<br>te Exp<br>eport<br>Peak I<br>Peak I<br>egration<br>2.56<br>2.06<br>1.56<br>1.06   | XT:     0.77     min       idow:     10.0     sec       pected RT:     No     v       Largest Peak     Midth:     3       Midth:     3     poin       Height:     0.00     on       Parameters   | Area: 7.257e5, Heig<br>3e5<br>2e5<br>1e5<br>0e0                  | o.1 0.2 0.3                                | 90 min  | 0  | 80                         | _                    |               | _                    | Area: 7.21                    | 16e5, Height: 3<br>3e5<br>2e5<br>1e5 | .939e5, RT: 0.                 | 30 min                                     |          | 0.7 0.8 0.5               | 9 1.0 1.1         |                 |                |             |      |
| cted F<br>If Win<br>te Exp<br>eport<br>Peak N<br>Peak N<br>Peak N<br>egration<br>2.5<br>2.0<br>1.5   | XT:     0.77     min       idow:     10.0     sec       pected RT:     No     v       Largest Peak     Midth:     3       Midth:     3     poin       Height:     0.00     on       Parameters   | Area: 7.257e5, Heig<br>3e5<br>2e5<br>1e5<br>0e0                  | o.1 0.2 0.3                                | 90 min  | 0  | 80                         | _                    |               | _                    | Area: 7.21                    | 16e5, Height: 3<br>3e5<br>2e5<br>1e5 | .939e5, RT: 0.                 | 30 min                                     |          | 0.7 0.8 0.5               | 9 1.0 1.1         |                 |                |             |      |



### **Sensitivity Data**

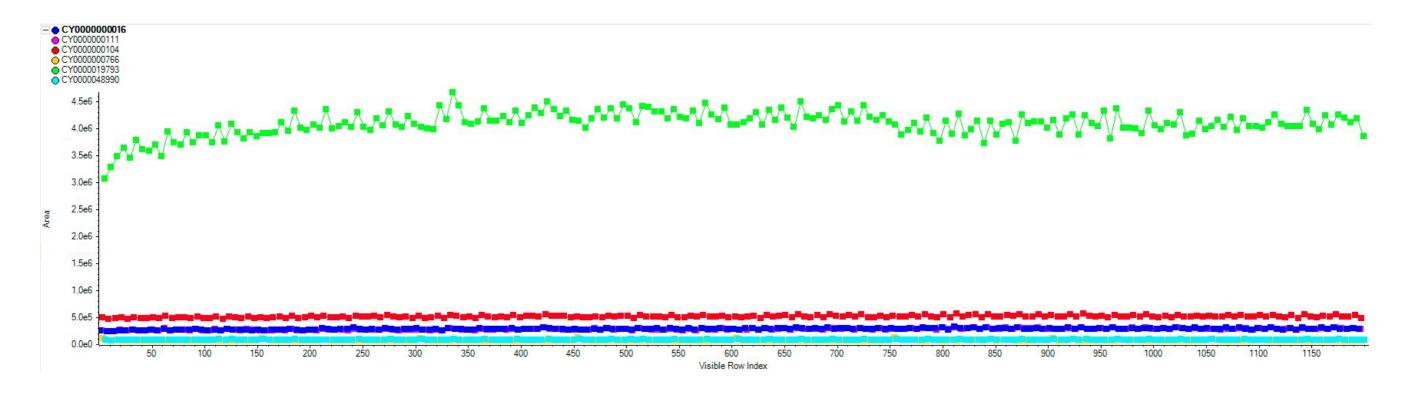
#### Example over a 6 week period





### **System Robustness Data**

Run over 200 injections





### **TripleTOF Data Workflow**

TTOF assistant

#### Set-up Application

| Instrument: | TTofM01      | Labsys:     | Live - Synchronise                             | 1 |
|-------------|--------------|-------------|--|---|
| Sample Ana  | alysis Tasks | Other Tasks |  |   |
| Pending     | 9            |             |  |   |
| Allocate    | ed:          |             |  | _ |
| Job         | Procedure    | e Status    |  |   |
| 156783      | P078         | TBD         | Show Details Archive Results Return To Pending |   |
| 156682      | P086         | TBD         | Show Details Archive Results Return To Pending |   |
| \$56582     | P097         | TBD         | Show Details Archive Results Return To Pending |   |
| 156410      | P086         | TBD         | Show Details Archive Results Return To Pending |   |
| 156304      | P086         | TBD         | Show Details Archive Results Return To Pending |   |
| 156297      | P085         | TBD         | Show Details Archive Results Return To Pending |   |
| 156224      | P086         | TBD         | Show Details Archive Results Return To Pending |   |
| 156194      | P086         | TBD         | Show Details Archive Results Return To Pending |   |
| 156128      | P069A        | TED         | Show Details Archive Results Return To Pending |   |
| 156125      | P086         | TBD         | Show Details Archive Results Return To Pending |   |
| 156096      | P078         | TBD         | Show Details Archive Results Return To Pending |   |
| 156076      | P069A        | TBD         | Show Details Archive Results Return To Pending |   |
| 156024      | P086         | TBD         | Show Details Archive Results Return To Pending |   |
| 155954      | P086         | TBD         | Show Details Archive Results Return To Pending |   |
| 155949      | P086         | TBD         | Show Details Archive Results Return To Pending |   |
| 155823      | P086         | TED         | Show Details Archive Results Return To Pending |   |
| 155820      | P085         | TED         | Show Details Archive Results Return To Pending |   |

#### Data extraction within the same application

| 4Q1 | Unrequested |                         |             |  |               |          |              |                 |                  |   |
|-----|-------------|-------------------------|-------------|--|---------------|----------|--------------|-----------------|------------------|---|
|     | Nethod N    | ame: DQ Stims Starter N | lethod dam  |  |               |          |              |                 |                  |   |
|     |             | Sample Id               | Sample Name | lie  | Date          | Tine     | Compound 18  | laz             | Atenative Mass   | * |
|     |             | AUSSTREAM INDIAL        | 0JM         | 156783/CPL/red_AQU/cd_156783_CF_14_Wel_AL_inc          | 31.1.ly 2020  | 194646   | Z CY80000004 | 268191 ± 0.010  |                  | 1 |
|     |             | A15678312,Weil,A1       | 450min      | 156789(2P, incl., AQUIdd, 156789, CF, 12, Nol, AQ, inc | 31. July 2020 | 194921   | V COMMAN     | 285,077 + 0,010 |                  | - |
|     | E G         | AUS678311, Weil, AL     | 310min      | 1567831CPLinc8_AQ0Uob_156783_CF_11_Wel_AL_inc          | 31 July 2020  | 1951.56  | 2.510000045  | 262477124624    |                  |   |
|     | 0.0         | AUSSTEELD, Well, AL     | 150min      | 156763/CPL/rel_AQUUeb_156763_CF_10_Wel_AL_inc          | 31. July 2020 | 195432   |              |                 |                  |   |
|     |             | A1567E09,Well,A1        | 50min       | 1567EBICPLineBLACEUob, 1567EB, CF, OB, NeBLALLine      | 31. July 2020 | 195707   |              |                 |                  |   |
|     |             | ACSETERIE, No., AL      | 00min       | 15671B1CP1, ind, AQ0306, 15671B1, CF, 08, Not, AL, inc | 31 July 2020  | 195941   |              |                 |                  |   |
|     |             | A1967EE13_Well,A1       | CH          | 1567EBCPL/w8_AQUU0_1567EB_CF_13_W6LAL/rc               | 31. My 2020   | 200218   |              |                 |                  |   |
|     |             | Sample Id               | Sample Name | File   | Date          | Tine     | Compound 18  | Tate            | Alternative Mass |   |
|     |             | A1567814,Wel,42         | 0.M         | 1567EBCB, ind, 4QU/db, 1567EB_C6_14, Well, 42, in      | 31. July 2020 | 200454   | CY000000004  | 268191 : 0.010  |                  |   |
|     |             | A1567E12,Wel,42         | 450min      | 156769.00 ind 400.00 156769.06 12 Wel 42 in            | 31 July 2020  | 20725    | V CYONNOUS   | 255.167 = 0.010 |                  |   |
|     |             | ADSTRUCTION AD          | 300min      | 156783.C61, indt, 401, bb, 156783, C6, 11, WHI, 42, in | 31.14y 2020   | 201005   | E. stansmin  | Plants, 1 Anth  | _                |   |
|     | CG CG       | A1567E10_Wei_A2         | 150min      | 196787.C0, ind, 4Q0,66, 196783, C5, 10, Wel, 42, in    | 31. July 2020 | 201241   |              |                 |                  |   |
|     |             | A15676309_Wel_A2        | 50min       | 1567E7.CG, ind, AQUAb, 1567E3, CG, OY, WHI, A2, in     | 31. July 2030 | 201516   |              |                 |                  |   |
|     |             | ASS7808, Wei, A2        | 00min       | 156783.CGI, indij.AQCU.eb, 156783_CG_08, Well, A2 in   | 31. July 2020 | 201752   |              |                 |                  |   |
|     |             | A1967633_Weil_A2        | CH          | 1567EBICO, ind AQUUS 1567EB_CO_13_Web_A2_in            | 31. July 2020 | 20,29,28 |              |                 |                  |   |
|     |             | Sample Id               | Sample Name | Re   | Date          | Tine     | Compound Id  | Taxe            | Alternative Mass |   |
|     |             | ADSITEDA_Well_A3        | 0.M         | 1567EBICH_IndE_AQUIde_1567EB_CH_1A_Wel_A3_in           | 31.hdy2020    | 20,29,05 | CY900000004  | 268191 ± 0.010  | 1000             |   |
|     |             | A15678312, Well, A3     | 450min      | 1567E3 CH_ind_AQUAb_1567E3_CH_12_Wel_A3_in             | 31. July 2020 | 202541   | 7 CYNNINCLED | 384.167 ± 0.000 |                  |   |
|     | E CH        | A15676311,Well,A3       | 310min      | 1967ERCH_indEAQUUde_1967EE_CH_11_WWI_A3_in             | 31.)øy 2020   | 20.28:17 | -            |                 |                  |   |
|     | 1 0         | A1567830, Well, A3      | 150min      | 156783.CH_indl_AQUUdb_156783_CH_10_Wel_A3_in           | 31.hJy 2020   | 203053   |              |                 |                  |   |
|     |             | A25676309_Wel_A3        | 50min       | 1567EBICH_ind_AQUIdb_1567EB_CH_09_Wel_A3_in            | 31.July 2020  | 2033.28  |              |                 |                  |   |
|     |             | ALSETERE, Weil, AD      | 00min       | 1567ERCH_Ind_AQUUd_1567EB_CH_0B_Wel_A3_in              | 31.1dy 2020   | 203604   |              |                 |                  |   |
|     |             | AUSS/TELS Well AS       | CH          | 196763 CH, Ind. AQUIX6 196763 CH 13 Well A3 In         | 31 July 2020  | 2038-40  |              |                 |                  |   |



### CAS

#### Chromatogram Analysis System





### Labsys Calculated Data

### An example of Clearance data

| de<br>پس)                           | 155432<br>CY000000047<br>dextromethorphan<br>A1<br>0.5<br>Lint<br>nin/mg<br>) SE(CLir                          | Strain<br>Sex<br>Protein Sou<br>Protein Typ<br>Batch   |  |   | Mixed<br>UNSPECIFIED<br>Liver<br>Microsomes<br>C38291   |  | Protein Conc. (mg/mL)<br>Co-Factor                             |   | 1<br>ADPH  |  |  |   |   |   |   |  |  |  |  |   |
|-------------------------------------|--|--|--|---|---|--|--|---|--|--|--|---|---|---|---|--|--|--|--|---|
| de<br>μ <b>Μ)</b><br>(μ/mi<br>(μ/mi | dextromethorphan<br>A1<br>0.5<br>Lint<br>nin/mg  | Protein Sou<br>Protein Typ   |  |   | Liver<br>Microsomes   |  | Co-Factor  |   |  |  |  |   |   |   |   |  |  |  |  |   |
| <b>LM)</b><br>CLi<br>(μl/mi         | A1<br>0.5<br>Lint<br>nin/mg  | Protein Typ  |  |   | Microsomes  | ;  |  | NA  |  |  |  |   |   |   |   |  |  |  |  |   |
| CLi<br>(µl/mi<br>(t½))              | 0.5<br>Lint<br>nin/mg  |  | e  |   |   | ;  | î  |   | NOPH   |  |  |   |   |   |   |  |  |  |  |   |
| CLi<br>(µl/mi<br>(t½))              | Lint<br>nin/mg   | Batch  |  |   | C20201  |  | Buffer   | KPO4/2  | mM MgCl2   | ۴J   |  |   |   |   |   |  |  |  |  |   |
| (μl/mi<br>(t½))                     | nin/mg   |  |  |   | 038291  |  | Termination Solution   | M   | eOH  |  |  |   |   |   |   |  |  |  |  |   |
| (μl/mi<br>(t½))                     | nin/mg   |  |  |   |   |  |  |   |  | 5 -  |  |   |   |   |   |  |  |  |  |   |
| (t½))                               |  |  |  |   | Control Accepta   | ance Criteria  |  |   |  | - t  |  |   |   |   |   |  |  |  |  |   |
|                                     |  |  | Decurre  | Lower<br>Bounds   | Upper Bounds  | Acceptable   | Fitting Choice   |   |  | 4  |  |   |   |   |   |  |  |  |  |   |
| .00   01                            |  | ) n<br>5   | R square<br>0.993  | 36  | 60  | No   | Auto   | _   |  | •1   |  |   |   |   |   |  |  |  |  |   |
|                                     | 1.7 1.51   |  | 0.555  | 50  | 00  | NO   | Auto   |   |  |  |  |   |   |   |   | •  |  |  |  |   |
|                                     |  |  |  |   | Timepoint   |  |  | 1   |  | 3 -  |  |   |   |   |   |  |  |  | -  |   |
| RT S/N R                            | Ratio Area Cm  | d IS Area  | Ratio  | %0 min  | excluded  |  | Warning  |   |  | _  |  |   |   |   |   |  |  |  |  |   |
| .54 315                             | 1574 31846   | 727767   | 0.438  | 100   |   |  |  | 1   |  | ji ji  |  |   |   |   |   |  |  |  |  |   |
| .54 204                             | 0482 25945   | 736420   | 0.352  | 80.5  |   |  |  | ]   |  | -E 2   |  |   |   |   |   |  |  |  |  |   |
| .54 215                             | 18636 18636  | 740415   | 0.252  | 57.5  |   |  |  | ]   |  | Ĕ  |  |   |   |   |   |  |  |  |  |   |
|                                     |  |  | 0.152  | 34.7  |   |  |  |   |  | ă  |  |   |   |   |   |  |  |  |  |   |
| .54 145                             | 1557 78270   | 741973   | 0.105  | 24.1  |   |  |  |   |  | <b>2</b> 1   |  |   |   |   |   |  |  |  |  |   |
|                                     |  |  |  |   |   |  |  | II •  | Percent  | 2  |  |   |   |   |   |  |  |  |  |   |
|                                     |  |  |  |   |   |  |  | 11  | Remaining  | ď,   |  |   |   |   |   |  |  |  |  |   |
| .54 26                              | 272  | 740024   | 0.000504   | 0.115   |   |  |  | •   | Omitted points   | <b>-</b> "1  |  |   |   |   |   |  |  |  |  |   |
|                                     |  | _  | -  |   |   |  |  | 11  |  |  |  |   |   |   |   |  |  |  |  |   |
| 1                                   |  |  |  |   | NA  |  |  | 11  |  | -1 -   |  |   |   |   |   |  |  |  |  |   |
|                                     |  |  |  |   |   |  |  |   | Control  |  |  |   |   |   |   |  |  |  |  |   |
| 2.237 > 171.1                       | 167 +  | 0.031662418  |  | QC%   | NA  |  |  |   |  |  |  |   |   |   |   |  |  |  |  |   |
| ernal Standar                       | ird  |  |  |   |   |  |  |   |  | -2 -   |  |   |   |   |   |  |  |  |  |   |
| SD RS                               | RSD Warnin   | 1  |  |   |   |  |  |   |  |  |  |   |   |   |   |  |  |  |  |   |
| 966 1.0                             | .07  |  |  |   |   |  |  | -   |  |  |  |   |   |   |   |  |  |  |  |   |
| t of range.                         |  |  |  |   |   |  |  |   |  | -3 -   | 5  | 10  | 15  | 20  | 25  | 20   | 35   | 40   | 45   | 50  |
|                                     |  |  |  |   |   |  |  | 1   |  | U U  |  |   |   |   |   |  |  |  |  |   |
| .5<br>.5<br>.5<br>.5<br>.5          | 4 21<br>4 18<br>4 14<br>4 14<br>4 24<br>4 24<br>4 24<br>4 24<br>100<br>237 > 171.<br>mal Standa<br>0 F<br>56 1 | 4         21503         186365           4         18280         114229           4         14557         78270           4         14557         78270           4         268         373           4         40217         336010           Ion         ±           237 > 171.167         +           rnal Standard         D           8SD         Warning           56         1.07 | 4         21503         186365         740415           4         18280         114229         751696           4         14557         78270         741973           4         268         373         740034           4         40217         336010         752560           Ion         ±         K VALUE           237 > 171.167         +         0.031662418           inal Standard         0         RSD           Varning         1.07         + | 4     21503     186365     740415     0.252       4     18280     114229     751696     0.152       4     14557     78270     741973     0.105       4     14557     78270     741973     0.105       4     268     373     740034     0.000504       4     40217     336010     752560     0.446       Ion     ±     K VALUE       237 > 171.167     +     0.031662418       imal Standard | 4         21503         186365         740415         0.252         57.5           4         18280         114229         751696         0.152         34.7           4         14557         78270         741973         0.105         24.1           4         14557         78270         741973         0.105         24.1           4         268         373         740034         0.000504         0.115           4         40217         336010         752560         0.446         102           Ion         ±         K VALUE         QC Ratio         QC Ratio           237 > 171.167         +         0.031662418         QC%           mal Standard              0         RSD         Warning | 4     21503     186365     740415     0.252     57.5       4     18280     114229     751696     0.152     34.7       4     14557     78270     741973     0.105     24.1       4     14557     78270     741973     0.105     24.1       4     268     373     740034     0.000504     0.115       4     40217     336010     752560     0.446     102       1on     ±     K VALUE     QC Ratio     NA       237 > 171.167     +     0.031662418     0     Min/High<br>QC%     NA       0     RSD     Warning     Warning     0     Warning | 4       21503       186365       740415       0.252       57.5 | 4       21503       186365       740415       0.252       57.5         4       18280       114229       751696       0.152       34.7         4       14557       78270       741973       0.105       24.1         4       268       373       740034       0.000504       0.115         4       40217       336010       752560       0.446       102         Ion $\pm$ K VALUE       QC Ratio       NA         237 > 171.167 $+$ 0.031662418       QC%       NA         9       RSD       Warning       Warning       Mark | 4       21503       186365       740415       0.252       57.5 | 4       21503       186365       740415       0.252       57.5 | 4       21503       186365       740415       0.252       57.5 | 4       14557       78270       741973       0.105       24.1 | 4       14557       78270       741973       0.105       24.1 | 4       14557       78270       741973       0.105       24.1 | 4       14557       78270       741973       0.105       24.1       1         4       14557       78270       741973       0.105       24.1       1       1         4       268       373       740034       0.000504       0.115       1       1       1         4       40217       336010       752560       0.446       102       1       1       1         10n       ±       K VALUE       QC Ratio       NA       1       1       1       1         237 > 171.167       +       0.031662418       QC%       NA       0       0       1       1         11       Control       Control | 4       14557       78270       741973       0.105       24.1       1         4       14557       78270       741973       0.105       24.1       1       1         4       268       373       740034       0.000504       0.115       1       1       1         4       40217       336010       752560       0.446       102       1       1       1         1on       ±       K VALUE       QC Ratio       NA       1       1       1       1         237 > 171.167       +       0.031662418       0 Min/High NA       0       0       1 <td>4       14557       78270       741973       0.105       24.1       Image: Constraint of the constraint of</td> <td>4       14557       78270       741973       0.105       24.1       Image: Constraint of the constraint of</td> <td>4       14557       78270       741973       0.105       24.1       0         4       14557       78270       741973       0.105       24.1       0         4       268       373       740034       0.000504       0.115       0         4       40217       336010       752560       0.446       102       0         1on       ±       KVALUE       QC Ratio       NA       0       0         237 &gt; 171.167       +       0.031662418       0       0/min/High       NA       0         237 &gt; 171.167       +       0.031662418       0       0/min/High       NA       Control       Control         0       RSD       Warning      </td> <td>4       14557       78270       741973       0.105       24.1      </td> | 4       14557       78270       741973       0.105       24.1       Image: Constraint of the constraint of | 4       14557       78270       741973       0.105       24.1       Image: Constraint of the constraint of | 4       14557       78270       741973       0.105       24.1       0         4       14557       78270       741973       0.105       24.1       0         4       268       373       740034       0.000504       0.115       0         4       40217       336010       752560       0.446       102       0         1on       ±       KVALUE       QC Ratio       NA       0       0         237 > 171.167       +       0.031662418       0       0/min/High       NA       0         237 > 171.167       +       0.031662418       0       0/min/High       NA       Control       Control         0       RSD       Warning | 4       14557       78270       741973       0.105       24.1 |



### **TTOF Data VS Triple Quadrupole**

Comparing control data

| Compound in Human<br>Hepatocytes or Microsomes | <b>Triple Quadrupole</b><br>CL <sub>int</sub> (µl/min/mg) | <b>TTOF</b><br>CL <sub>int</sub> (µl/min/mg) |
|--|---|--|
| CY47   | 31.7  | 33.0   |
| CY132  | 6.06  | 6.18   |
| CY173  | 148   | 163  |

Sample injected on the TTOF and then re-injected on a Triple Quadrupole for a set of controls which resulted in near identical CL<sub>int</sub> values



### **Softspot Analysis**

Why the current interest?

- Generation of qualitative MS/MS data from same injection as quantitative MS CL data. Metabolite identification of top 3 metabolites.
- MS and MS/MS accuracy needs to be maintained below 2ppm for sufficient data quality. Minimum 10 data points need to be maintained in TOF MS mode to obtain sufficient quality data for quantitation.
- Data evaluation of qualitative data through Metabolite Pilot or 3<sup>rd</sup> Party vendor software



### Soft Spot Met ID

#### Using MetabolitePilot<sup>™</sup> Software

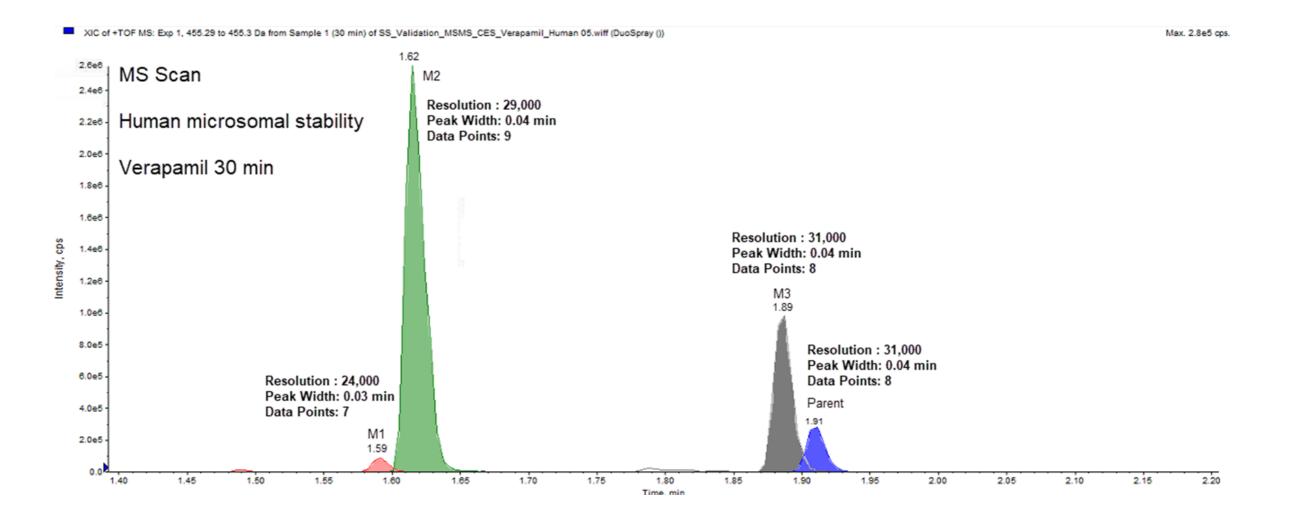
For each test compound in both species the 30 minute sample was compared against the 0 minute control using Metabolite Pilot software. The top 3 metabolite were reported.

| Peak ID                   | Name   | Formula  | Neutral Mass  | m/z  | ppm  | R.T. (min)  | Peak Area  | % Area  |                           |
|---------------------------|--|--|---|--|--|---|--|---|---------------------------|
|                           | Parent [M+H]+  | C27H38N2O4   | 454.2839  | 455.2912   | 1.7  | 1.91  | 4.74E+05   | 9.20  |                           |
| M1                        | Loss of C10H12O2 and CH2 [M+H]+  | C16H24N2O2   | 276.1842  | 277.1914   | 1.4  | 1.59  | 1.14E+05   | 2.20  | Human Verapamil           |
| M2                        | Loss of C10H12O2 [M+H]+  | C17H26N2O2   | 290.2000  | 291.2073   | 2.1  | 1.61  | 3.18E+06   | 61.3  |                           |
| M3                        | Loss of CH2 [M+H]+   | C26H36N2O4   | 440.2685  | 441.2757   | 2.2  | 1.89  | 1.42E+06   | 27.4  |                           |
|                           |  |  |   |  |  |   |  |   |                           |
| Peak ID                   | Name   | Formula  | Neutral Mass  | m/z  | ppm  | R.T. (min)  | Peak Area  | % Area  | _                         |
|                           | Parent [M+H]+  | C27H38N2O4   | 454.2832  | 455.2905   | 0.0  | 1.91  | 2.25E+05   | 6.30  |                           |
| M1                        | Loss of C10H12O2 and CH2 [M+H]+  | C16H24N2O2   | 276.1833  | 277.1906   | -1.6   | 1.59  | 1.80E+05   | 5.00  | Mouse Verapamil           |
| M2                        | Loss of C10H12O2 [M+H]+  | C17H26N2O2   | 290.1991  | 291.2064   | -1.0   | 1.61  | 2.15E+06   | 59.8  |                           |
| M3                        | Loss of CH2 [M+H]+   | C26H36N2O4   | 440.2673  | 441.2745   | -0.6   | 1.88  | 1.04E+06   | 28.9  |                           |
|                           |  |  |   |  |  |   |  |   |                           |
|                           |  |  |   |  |  |   |  |   |                           |
| Peak ID                   | Name   | Formula  | Neutral Mass  | m/z  | ppm  | R.T. (min)  | Peak Area  | % Area  |                           |
| Peak ID                   | Name<br>Parent [M+H]+  | Formula<br>C18H25NO  | Neutral Mass<br>271.1939  | <b>m/z</b><br>272.2011   | <b>ppm</b><br>0.9                                | <b>R.T. (min)</b><br>1.69                                 | Peak Area<br>6.87E+06  | % <b>Area</b><br>57.3                                 | Human                     |
| Peak ID<br>M1             |  |  |   |  |  | . ,   |  |   | Human                     |
|                           | Parent [M+H]+  | C18H25NO   | 271.1939  | 272.2011   | 0.9  | 1.69  | 6.87E+06   | 57.3  | Human<br>Dextromethorphan |
| M1                        | Parent [M+H]+<br>Loss of CH2 [M+H]+  | C18H25NO<br>C17H23NO   | 271.1939<br>257.1778  | 272.2011<br>258.1851   | 0.9<br>-0.6                                      | 1.69<br>1.37  | 6.87E+06<br>4.65E+06   | 57.3<br>38.8  |                           |
| M1<br>M2                  | Parent [M+H]+<br>Loss of CH2 [M+H]+<br>Loss of CH2 [M+H]+  | C18H25NO<br>C17H23NO<br>C17H23NO                                     | 271.1939<br>257.1778<br>257.1777  | 272.2011<br>258.1851<br>258.1849                                       | 0.9<br>-0.6<br>-1.2                              | 1.69<br>1.37<br>1.67                                      | 6.87E+06<br>4.65E+06<br>2.30E+05   | 57.3<br>38.8<br>1.90<br>2.00                          |                           |
| M1<br>M2                  | Parent [M+H]+<br>Loss of CH2 [M+H]+<br>Loss of CH2 [M+H]+  | C18H25NO<br>C17H23NO<br>C17H23NO                                     | 271.1939<br>257.1778<br>257.1777  | 272.2011<br>258.1851<br>258.1849                                       | 0.9<br>-0.6<br>-1.2                              | 1.69<br>1.37<br>1.67                                      | 6.87E+06<br>4.65E+06<br>2.30E+05   | 57.3<br>38.8<br>1.90                                  |                           |
| M1<br>M2<br>M3            | Parent [M+H]+<br>Loss of CH2 [M+H]+<br>Loss of CH2 [M+H]+<br>Oxidation [M+H]+                          | C18H25NO<br>C17H23NO<br>C17H23NO<br>C18H25NO2                        | 271.1939<br>257.1778<br>257.1777<br>287.1884                                    | 272.2011<br>258.1851<br>258.1849<br>288.1956                           | 0.9<br>-0.6<br>-1.2<br>-0.6                      | 1.69<br>1.37<br>1.67<br>1.74                              | 6.87E+06<br>4.65E+06<br>2.30E+05<br>2.46E+05                                 | 57.3<br>38.8<br>1.90<br>2.00                          | Dextromethorphan          |
| M1<br>M2<br>M3            | Parent [M+H]+<br>Loss of CH2 [M+H]+<br>Loss of CH2 [M+H]+<br>Oxidation [M+H]+<br>Name                  | C18H25NO<br>C17H23NO<br>C17H23NO<br>C18H25NO2<br>Formula             | 271.1939<br>257.1778<br>257.1777<br>287.1884<br>Neutral Mass                    | 272.2011<br>258.1851<br>258.1849<br>288.1956<br><b>m/z</b>             | 0.9<br>-0.6<br>-1.2<br>-0.6<br><b>ppm</b>        | 1.69<br>1.37<br>1.67<br>1.74<br><b>R.T. (min)</b>         | 6.87E+06<br>4.65E+06<br>2.30E+05<br>2.46E+05<br>Peak Area                    | 57.3<br>38.8<br>1.90<br>2.00<br><b>% Area</b>         | Dextromethorphan<br>Mouse |
| M1<br>M2<br>M3<br>Peak ID | Parent [M+H]+<br>Loss of CH2 [M+H]+<br>Loss of CH2 [M+H]+<br>Oxidation [M+H]+<br>Name<br>Parent [M+H]+ | C18H25NO<br>C17H23NO<br>C17H23NO<br>C18H25NO2<br>Formula<br>C18H25NO | 271.1939<br>257.1778<br>257.1777<br>287.1884<br><b>Neutral Mass</b><br>271.1942 | 272.2011<br>258.1851<br>258.1849<br>288.1956<br><b>m/z</b><br>272.2015 | 0.9<br>-0.6<br>-1.2<br>-0.6<br><b>ppm</b><br>2.1 | 1.69<br>1.37<br>1.67<br>1.74<br><b>R.T. (min)</b><br>1.69 | 6.87E+06<br>4.65E+06<br>2.30E+05<br>2.46E+05<br><b>Peak Area</b><br>1.70E+06 | 57.3<br>38.8<br>1.90<br>2.00<br><b>% Area</b><br>22.0 | Dextromethorphan          |



### **MS Scan**

#### Focusing on the resolution/number of data points





### **Future Plans**

What will the client expect in the future?

- Increased dynamic range (but probably TOF limitations!)
- Further use for MRM or TOF MS analysis for difficult compounds in the quantitative bioanalytical area
- Combination of rapid chromatography and TTOF technology to further reduce cycle times
- Integration of Echo MS to HRAM platform to give 1 second cycle times and no requirement for compound optimisation
- Increase use for quan/qual workflows
- Instrument requirements smaller foot print for future TOFs, improved sensitivity (always a requirement), reduced maintenance, improved control of 3<sup>rd</sup> party instruments (improve/remove ADD)
- Future plans will always be dependent on client demands, Cyprotex needs to continue to be front runners in adopting best in class technologies and software solutions



### Summary/Conclusions

Are TripleTOF<sup>®</sup> systems the future for HT-ADME/toxicity screening?



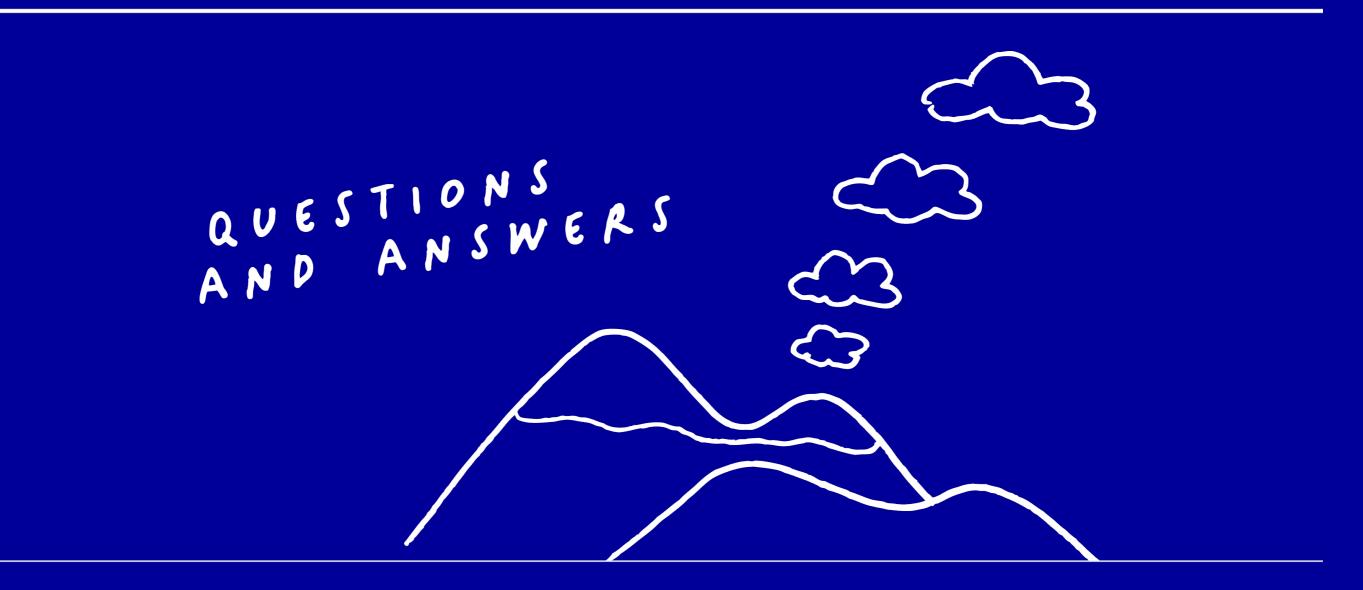
- HRAM MS is the way forward for HT-ADME/toxicity screening
- Delivery of quality data to ever demanding timelines requires the advantages that TOF can facilitate
- Increase access to real time qualitative data in parallel to quantitative data will continue to be a driver in drug discovery



### Acknowledgements

- HT Analytical Team at Cyprotex
- Anna Kerins (Associate Principal Scientist Metabolite profiling)
- Simon Wood (Head of Analytical Sciences)
- IS department at Cyprotex







#### Your contact:

Hollie Adey HT Analytical Senior Laboratory Supervisor

+44.(0)1625.505105 +44.(0)1625.505199 H.Adey@Cyprotex.com