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

Hollie Adey, Cyprotex, September 2020

Agenda

Overview of Cyprotex

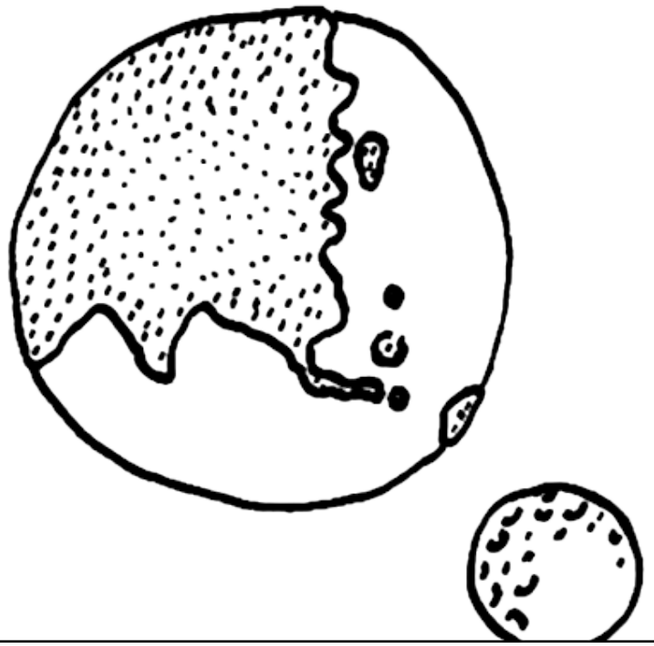
Overview of HT Processes

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

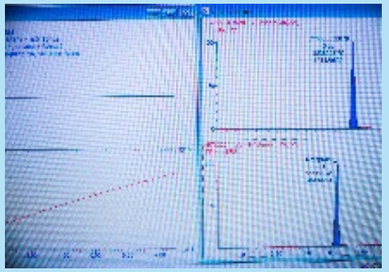
- Established in 1999, Cyprotex Limited was acquired by Evotec AG in 2016
- As part of the Evotec group, Cyprotex focuses on the ADME-Tox
- Our clients mainly focus on pharmaceutical research, however, we also support clients in the chemical, cosmetics and personal care, agrichemical and tobacco industries
- 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™) 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 compounds	5,864 plates made up	8,249 assays	5,804,813 samples measured	377,789 results generated
Compound receipt	**Assay plate preparation**	**Experimental set up and performance**	**Sample measurement**	**Result processing and QC**
				
11,109 test compound vessels	5,864 assay input plates	28,433 analysis plates	1,146,776 MS injections	4,431,234 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 have large cassette sizes
Decreased data size	Ability to re-extract data looking for adducts / alternatives masses / metabolites at anytime
No extra samples run due to CDS which increases run time	Addition of different IS then extract data using the one you want
Instant chromatographic data available	

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		Assay run on instrument in positive ionisation
Spectra and chromatography processed, if passes in-house criteria optimisation is copied in master database	**Day 2**	Data extracted on positive ion data, suitable data reported to client
		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.

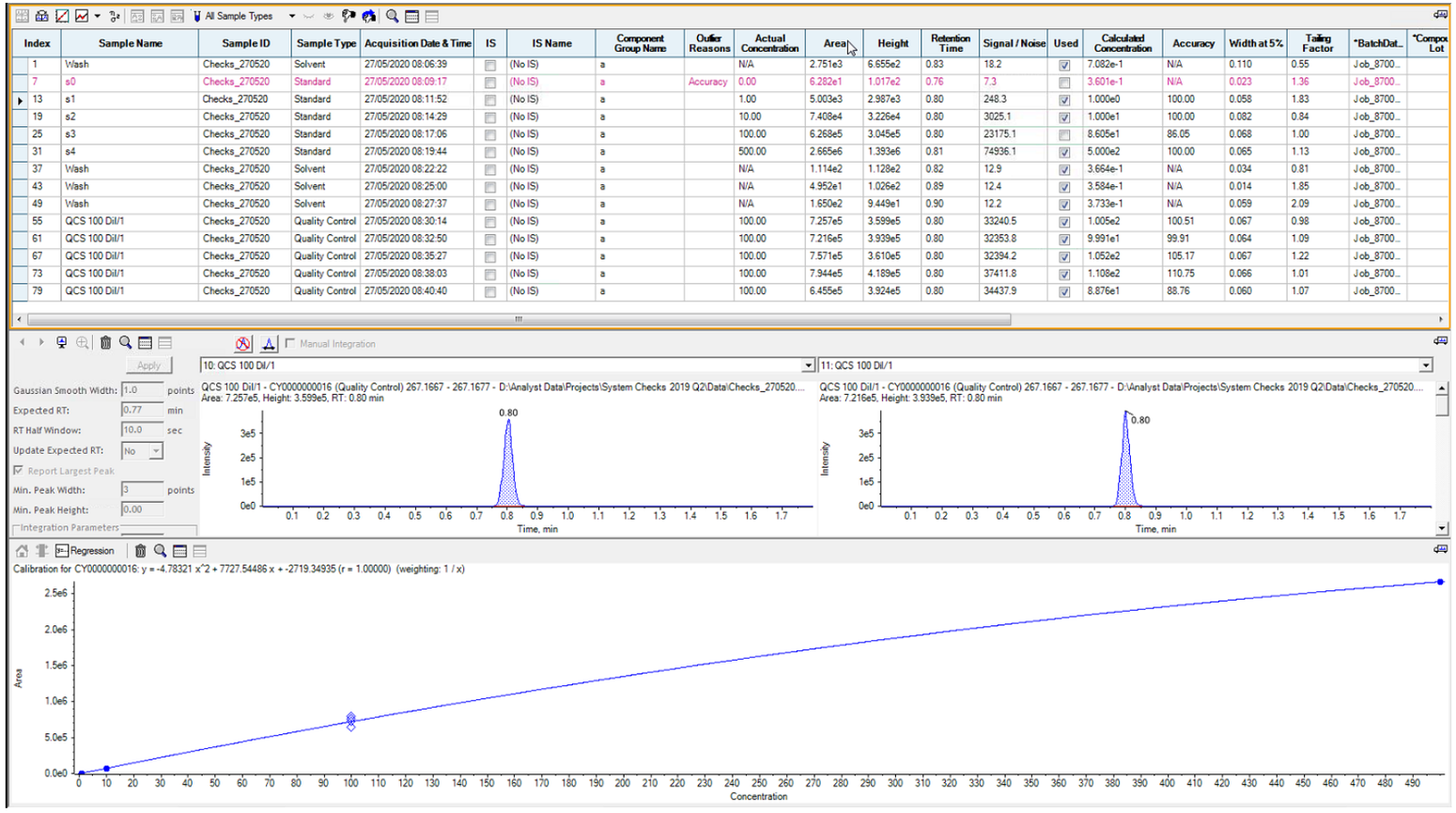
Maintenance and Performance Checks

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

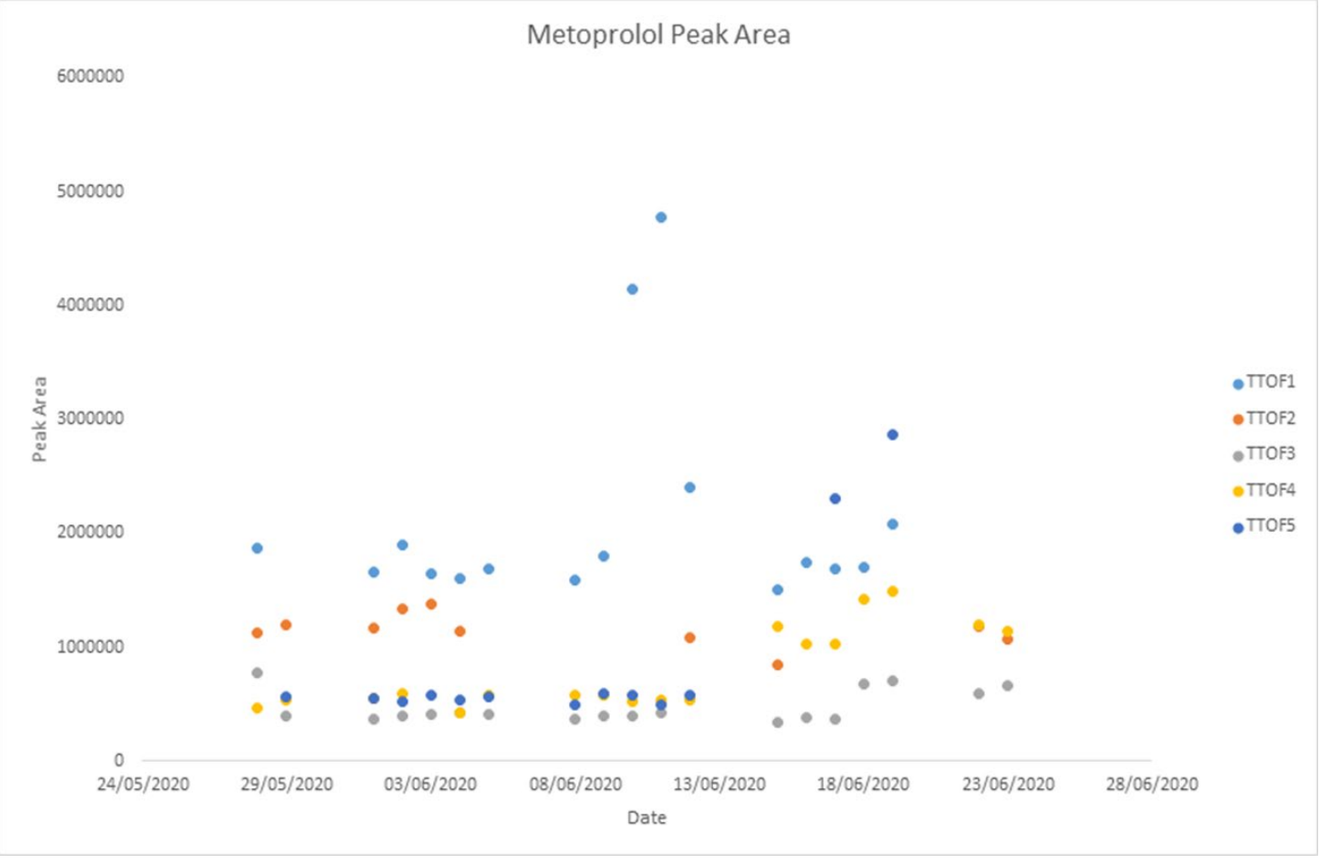
MultiQuant™ Software Data



Index	Sample Name	Sample ID	Sample Type	Acquisition Date & Time	IS	IS Name	Component Group Name	Outlier Reasons	Actual Concentration	Area	Height	Retention Time	Signal / Noise	Used	Calculated Concentration	Accuracy	Width at 5%	Tailing Factor	*BatchDat...	*Compo Lot
1	Wash	Checks_270520	Solvent	27/05/2020 08:06:39		(No IS)	a		N/A	2.751e3	6.655e2	0.83	18.2		7.082e-1	N/A	0.110	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	0.76	7.3		3.601e-1	N/A	0.023	1.36	Job_8700...	
13	s1	Checks_270520	Standard	27/05/2020 08:11:52		(No IS)	a		1.00	5.003e3	2.987e3	0.80	248.3		1.000e0	100.00	0.058	1.83	Job_8700...	
19	s2	Checks_270520	Standard	27/05/2020 08:14:29		(No IS)	a		10.00	7.408e4	3.226e4	0.80	3025.1		1.000e1	100.00	0.082	0.84	Job_8700...	
25	s3	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	0.068	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	0.065	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	0.034	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		3.584e-1	N/A	0.014	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		3.733e-1	N/A	0.059	2.09	Job_8700...	
55	QCS 100 Dil/1	Checks_270520	Quality Control	27/05/2020 08:30:14		(No IS)	a		100.00	7.257e5	3.599e5	0.80	33240.5		1.005e2	100.51	0.067	0.98	Job_8700...	
61	QCS 100 Dil/1	Checks_270520	Quality Control	27/05/2020 08:32:50		(No IS)	a		100.00	7.216e5	3.939e5	0.80	32353.8		9.991e1	99.91	0.064	1.09	Job_8700...	
67	QCS 100 Dil/1	Checks_270520	Quality Control	27/05/2020 08:35:27		(No IS)	a		100.00	7.571e5	3.610e5	0.80	32394.2		1.052e2	105.17	0.067	1.22	Job_8700...	
73	QCS 100 Dil/1	Checks_270520	Quality Control	27/05/2020 08:38:03		(No IS)	a		100.00	7.944e5	4.189e5	0.80	37411.8		1.108e2	110.75	0.066	1.01	Job_8700...	
79	QCS 100 Dil/1	Checks_270520	Quality Control	27/05/2020 08:40:40		(No IS)	a		100.00	6.455e5	3.924e5	0.80	34437.9		8.876e1	88.76	0.060	1.07	Job_8700...	

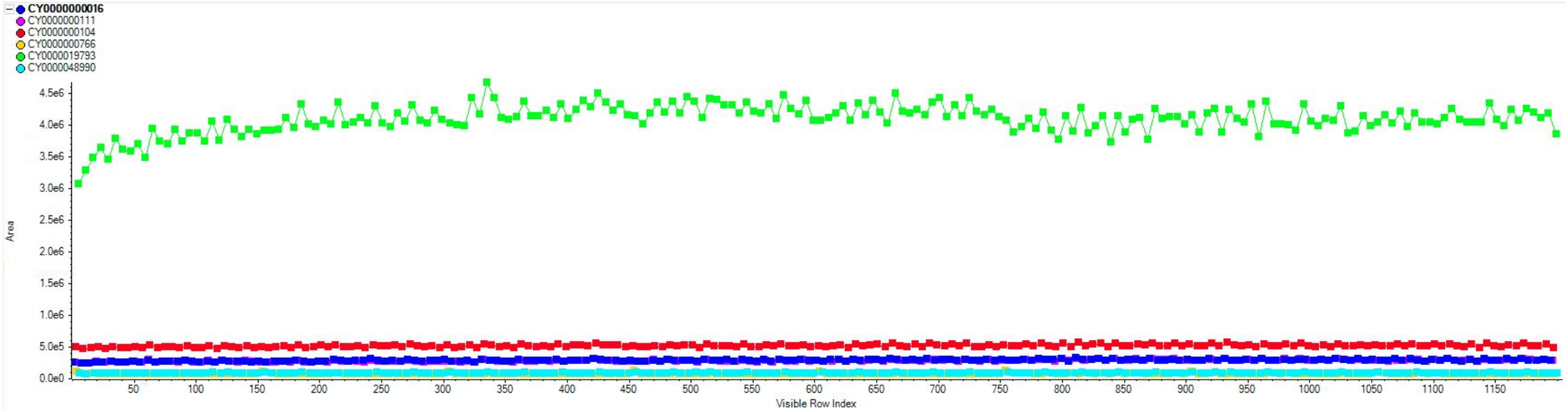
Sensitivity Data

Example over a 6 week period



System Robustness Data

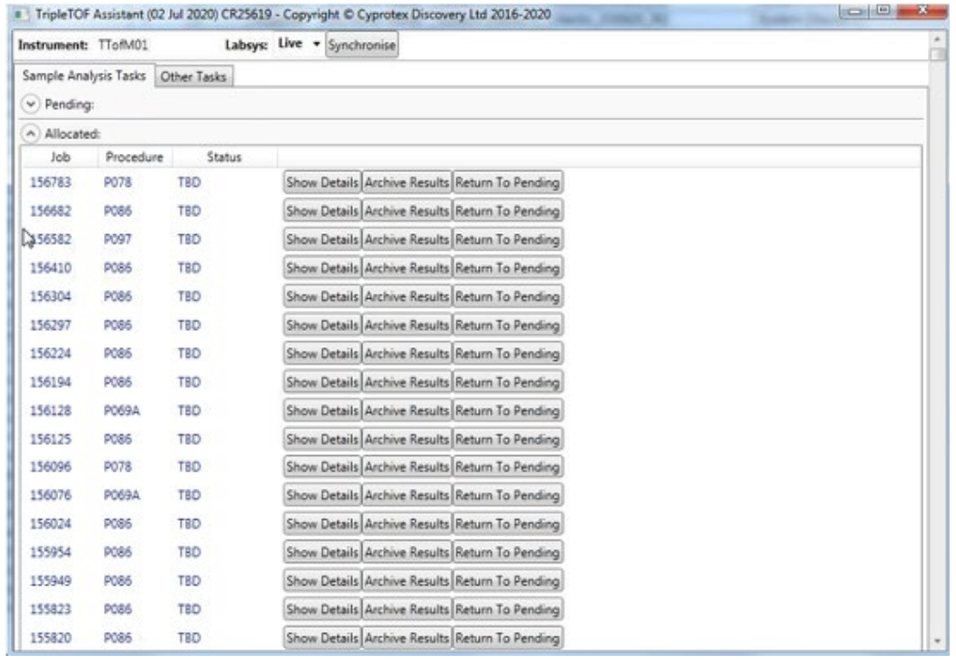
Run over 200 injections



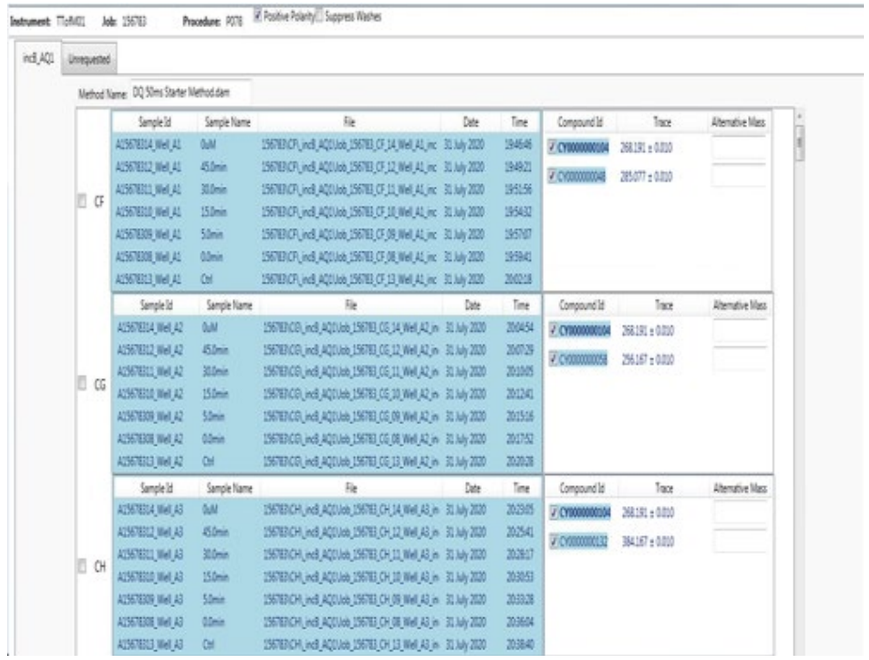
TripleTOF Data Workflow

TTOF assistant

Set-up Application

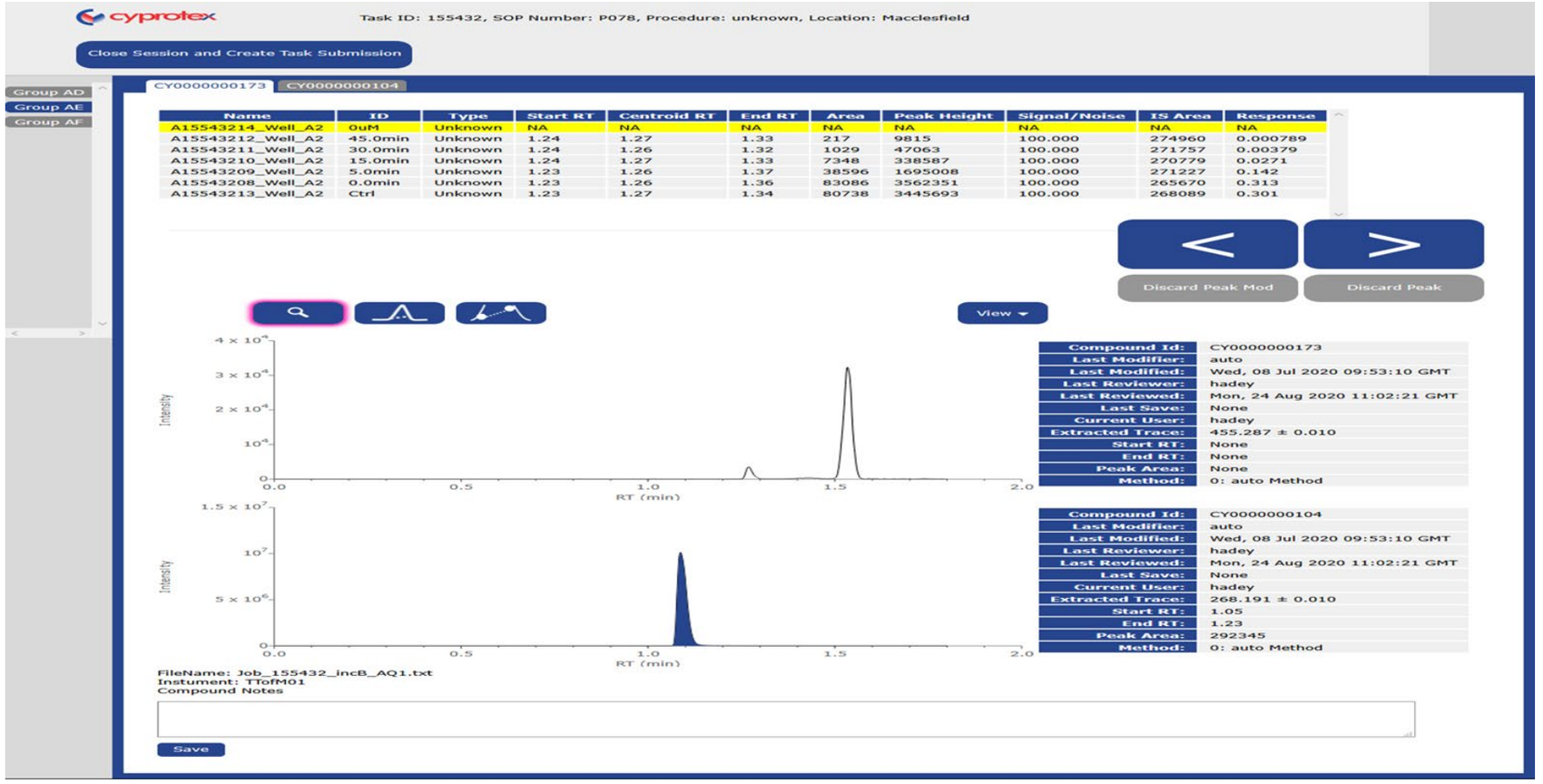


Data extraction within the same application



CAS

Chromatogram Analysis System



Labsys Calculated Data

An example of Clearance data

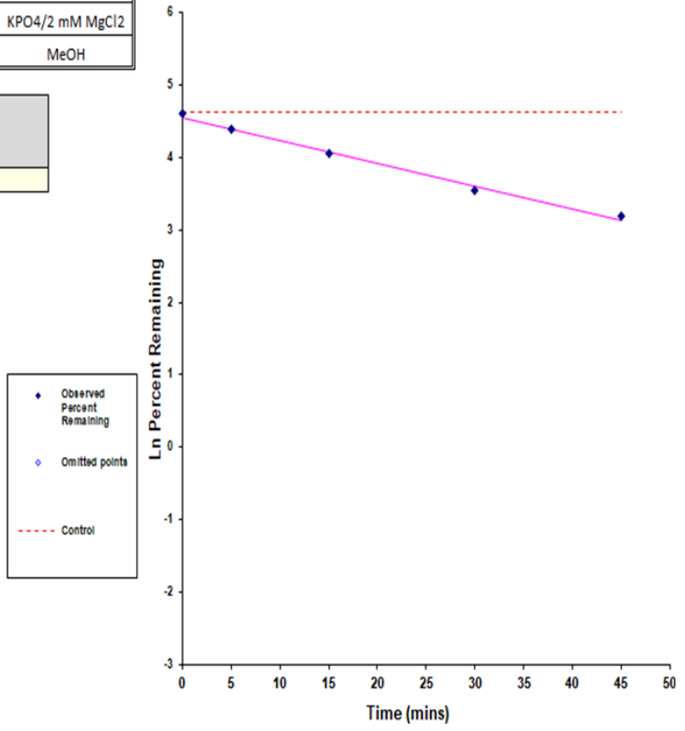
Study	BEN0001	**Species**	Human		
Job Number	155432	**Strain**	Mixed		
Cyprotex Id	CY0000000047	**Sex**	UNSPECIFIED	**Protein Conc. (mg/mL)**	1
Customer Id	dextromethorphan	**Protein Source**	Liver	**Co-Factor**	NADPH
Well	A1	**Protein Type**	Microsomes	**Buffer**	KPO4/2 mM MgCl2
Substrate Conc. (µM)	0.5	**Batch**	C38291	**Termination Solution**	MeOH

t ½ (min)	SE(t ½)	CLint (µl/min/mg)	SE(CLint)	n	R square	Positive Control Acceptance Criteria			Fitting Choice
						Lower Bounds	Upper Bounds	Acceptable	
21.9	1.05	31.7	1.51	5	0.993	36	60	No	Auto

Time (min)	RT	S/N Ratio	Area Cmpd	IS Area	Ratio	% 0 min	Timepoint excluded	Warning
0	0.54	31574	318464	727767	0.438	100		
5	0.54	20482	259457	736420	0.352	80.5		
15	0.54	21503	186365	740415	0.252	57.5		
30	0.54	18280	114229	751696	0.152	34.7		
45	0.54	14557	78270	741973	0.105	24.1		
0µM	0.54	268	373	740034	0.000504	0.115		
Ctrl	0.54	40217	336010	752560	0.446	102		
Reference RT	Ion		±	K VALUE		QC Ratio	NA	
0.54	272.237 > 171.167		+	0.031662418		0 Min/High QC%	NA	

Internal Standard			
Mean	SD	RSD	Warning
741552.1429	7966	1.07	

Control compound out of range.



TTOF Data VS Triple Quadrupole

Comparing control data

Compound in Human Hepatocytes or Microsomes	**Triple Quadrupole** CL_{int} (µl/min/mg)	**TTOF** CL_{int} (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_{int} 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 3rd Party vendor software

Soft Spot Met ID

Using MetabolitePilot™ 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
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

Human Verapamil

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
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

Mouse Verapamil

Peak ID	Name	Formula	Neutral Mass	m/z	ppm	R.T. (min)	Peak Area	% Area
	Parent [M+H]+	C18H25NO	271.1939	272.2011	0.9	1.69	6.87E+06	57.3
M1	Loss of CH2 [M+H]+	C17H23NO	257.1778	258.1851	-0.6	1.37	4.65E+06	38.8
M2	Loss of CH2 [M+H]+	C17H23NO	257.1777	258.1849	-1.2	1.67	2.30E+05	1.90
M3	Oxidation [M+H]+	C18H25NO2	287.1884	288.1956	-0.6	1.74	2.46E+05	2.00

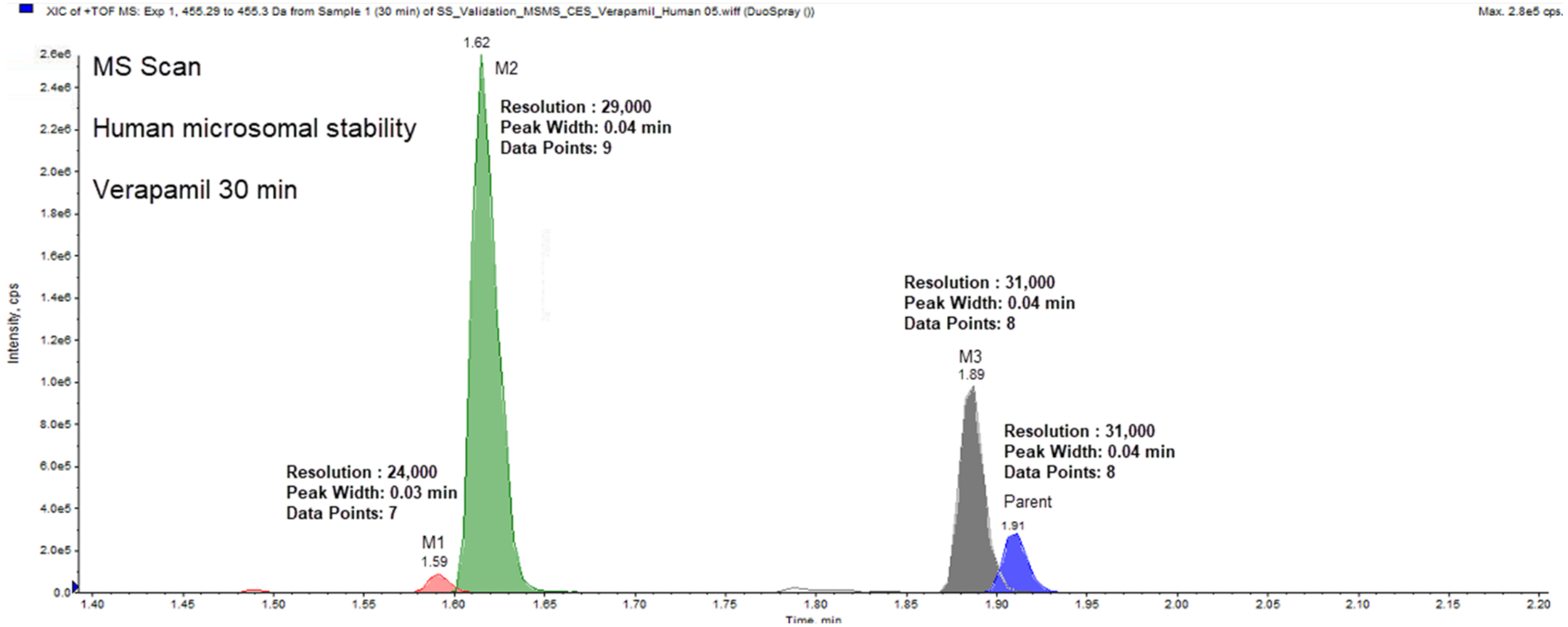
Human Dextromethorphan

Peak ID	Name	Formula	Neutral Mass	m/z	ppm	R.T. (min)	Peak Area	% Area
	Parent [M+H]+	C18H25NO	271.1942	272.2015	2.1	1.69	1.70E+06	22.0
M1	Loss of CH2 and CH2 [M+H]+	C16H21NO	243.1628	244.1701	2.1	1.36	4.59E+05	5.90
M2	Loss of CH2 [M+H]+	C17H23NO	257.1783	258.1856	1.4	1.38	5.25E+06	67.8
M3	Oxidation [M+H]+	C18H25NO2	287.1897	288.1970	4.2	1.75	3.29E+05	4.20

Mouse 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 3rd 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® 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

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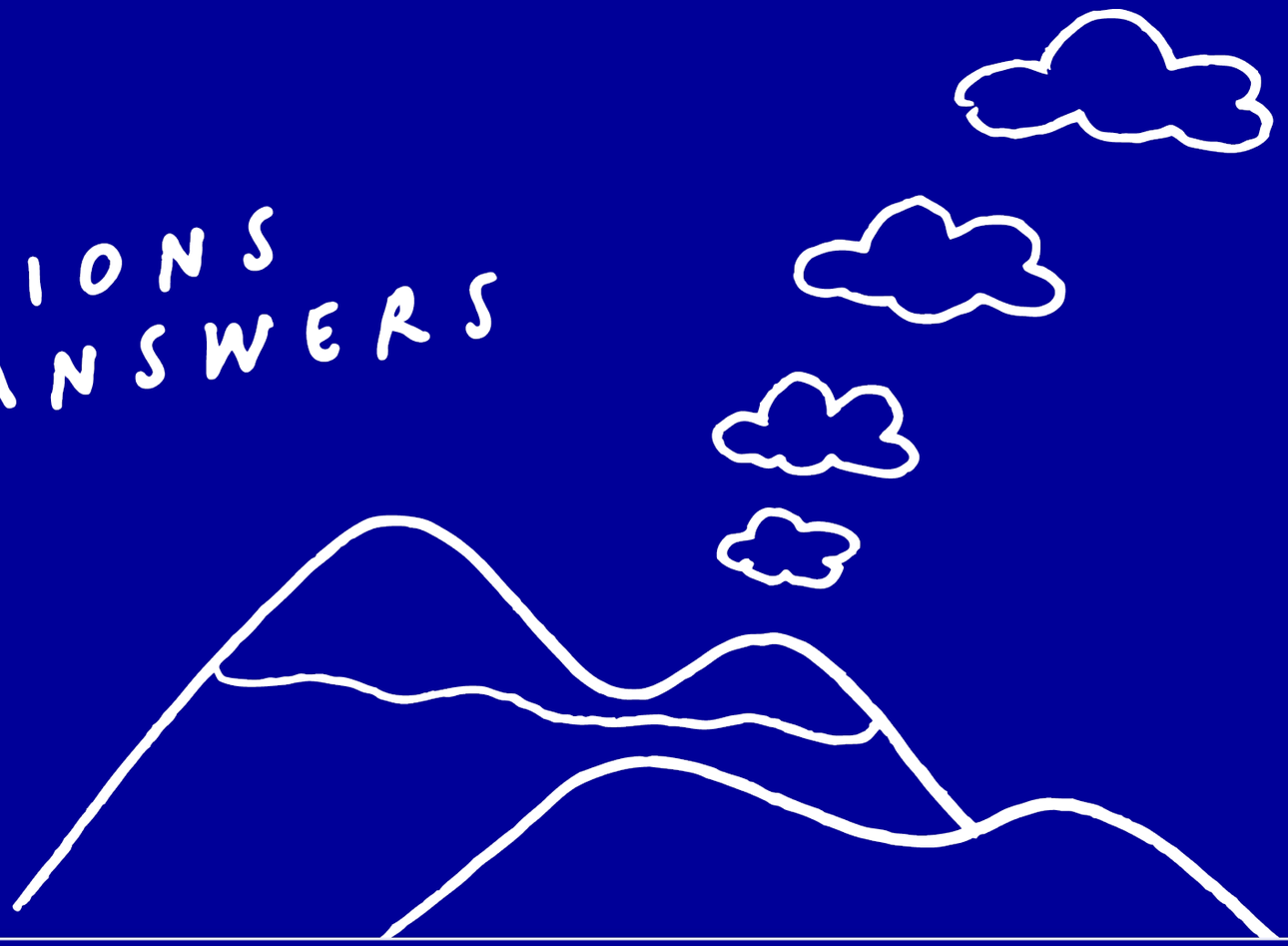
QUESTIONS AND ANSWERS



Your contact:

Hollie Adey
HT Analytical Senior Laboratory Supervisor

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