Outsourcing of Small molecule LC-MS bioanalytical methods: when the assay has to be adapted

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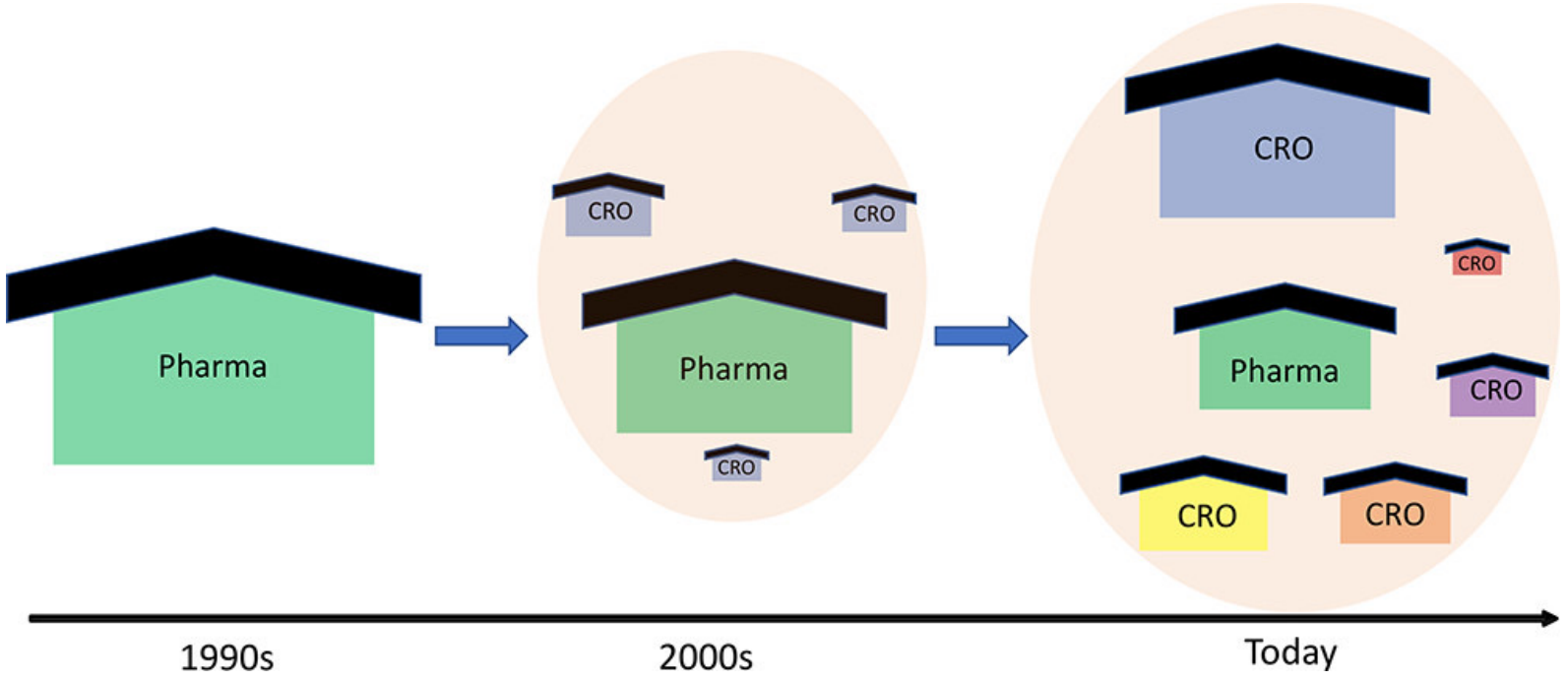
Current trend in outsourced bioanalytical activities



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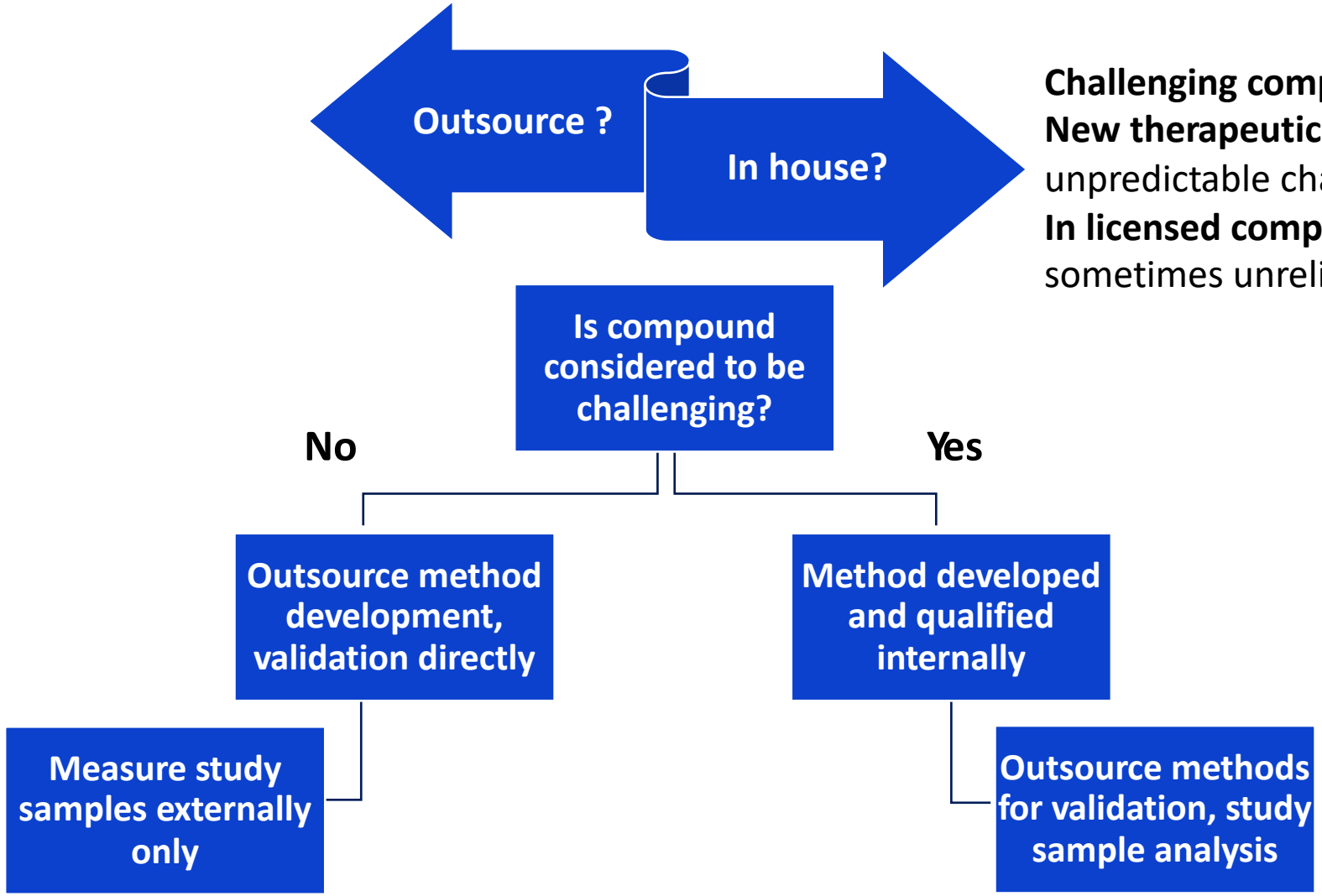
➢ **Objectives:**

→ Gain in flexibility (mainly big pharma)
→ Internal capacity saving – Focus on innovation (both big pharma and startup)
→ Cost saving (mainly startup)
→ Get expert support (mainly startup)

Small molecule bioanalytical outsourcing strategy at Roche



Challenging compounds:
New therapeutic classes with additional or unpredictable challenges
In licensed compounds with tight timelines and sometimes unreliable background information

Key factors to be considered during method development for an easily transferrable method

Experimental procedure	Robustness	Parameters qualified internally
• Least complex methods possible • Avoid unnecessary complex extractions • Avoid usage of “difficult to obtain” reagents or materials • Avoid using more rare equipment	• Test method thoroughly before transfer • Ensure room for sensitivity losses during transfers • Do not expect same performance at external lab	• Linearity • P/A (intra day) • Stability • Matrix effects • Carry-over • Selectivity and specificity

➢ **Objectives:**

→ Keep knowledge and expertise internally

→ Ensure efficient method transfers and validations

→ Avoid failures during method transfers

→ Meeting project timelines

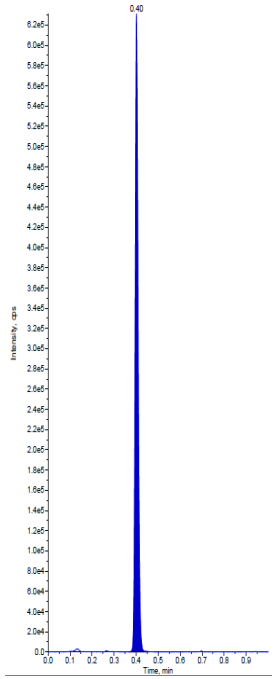
….. Despite this, some additional challenges can be encountered when outsourcing small molecule LCMS methods

Case example 1: Timelines - Lack of biotransformation data

In-licensed compound with tight project timelines

- In-licensed small molecule covalent inhibitor containing a reactive warhead
- Method was developed partially internally to ensure stability in biological samples
- Method validated at CRO prior to non-GLP study sample analysis and *in vivo* MetID data availability

Mouse plasma 2h



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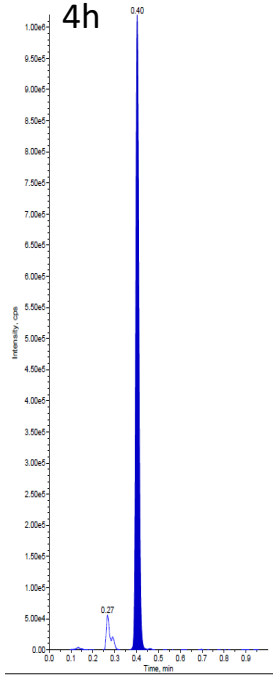

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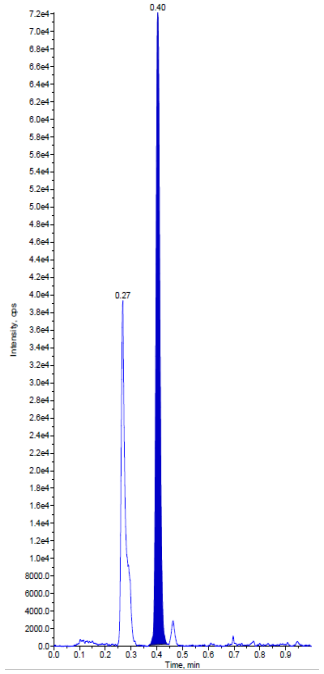
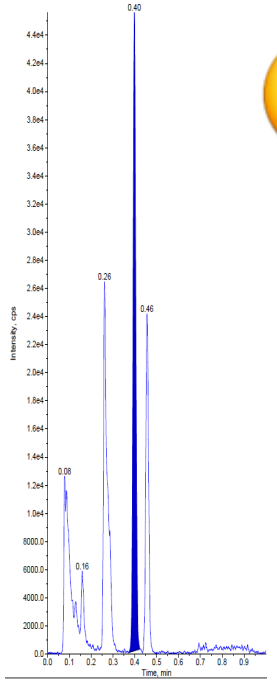


Mouse urine 0-24h



Time (min)	A (%)	B (%)	Flow (mL/min)
0.00	65.0	35.0	1.50
0.50	60.0	40.0	1.50
0.52	5.0	95.0	1.50
0.75	5.0	95.0	1.50
0.77	65.0	35.0	1.50

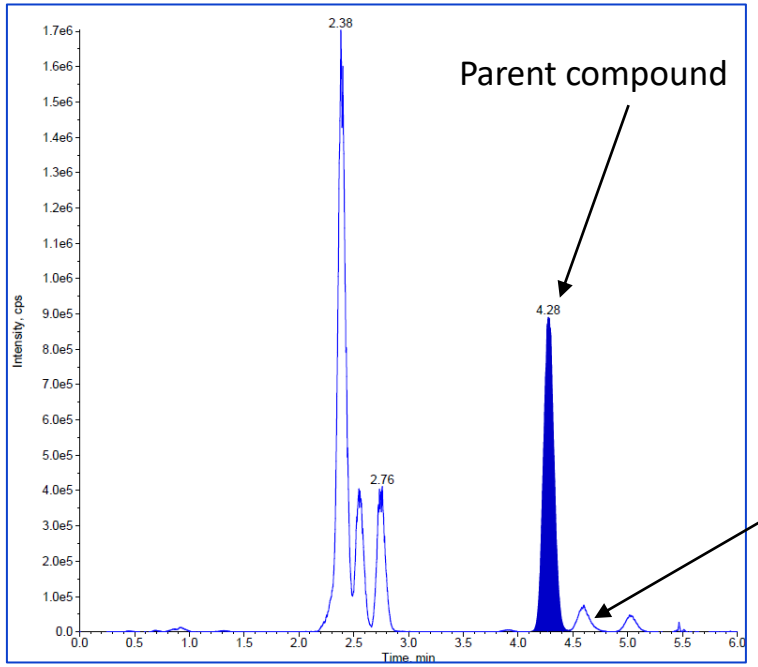
Initial gradient, good retention factor (k')
Column dimensions: 2.1x30mm, 1.7µm, RP

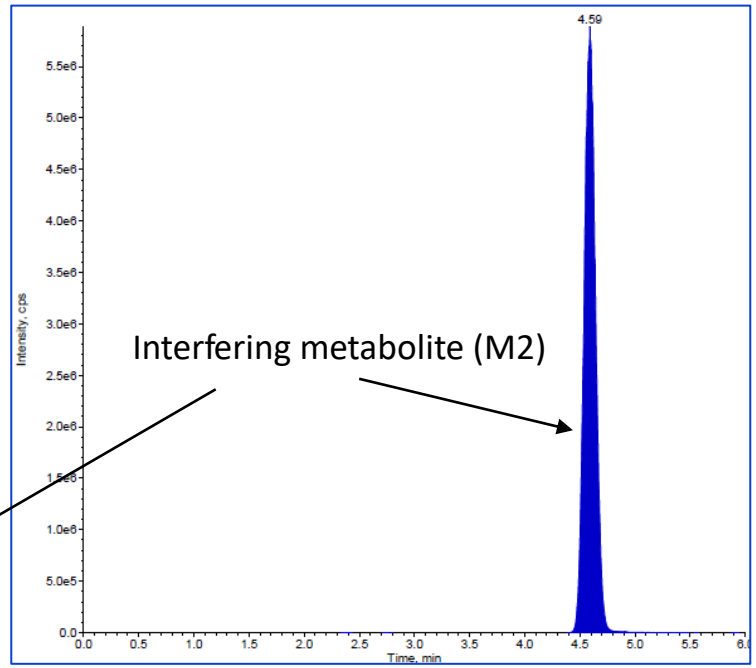
- Multiple closely related compounds responding at the same MRM transition than the parent drug in study samples
- Is specificity still given? Probably not in case of co-elution

Case example 1: Timelines - Lack of biotransformation data

In-licensed compound with tight project timelines

- In the meantime, in vivo metabolites were identified and could be tested for interferences in the BA method
- Initial validated method showed interferences coming from in source fragmentations of co-eluting, in vivo metabolites





Time (min)	A (%)	B (%)	Flow (mL/min)
0.00	72.0	28.0	0.750
5.00	70.0	30.0	0.750
5.04	5.0	95.0	0.750
5.50	5.0	95.0	0.750
5.54	72.0	28.0	0.750

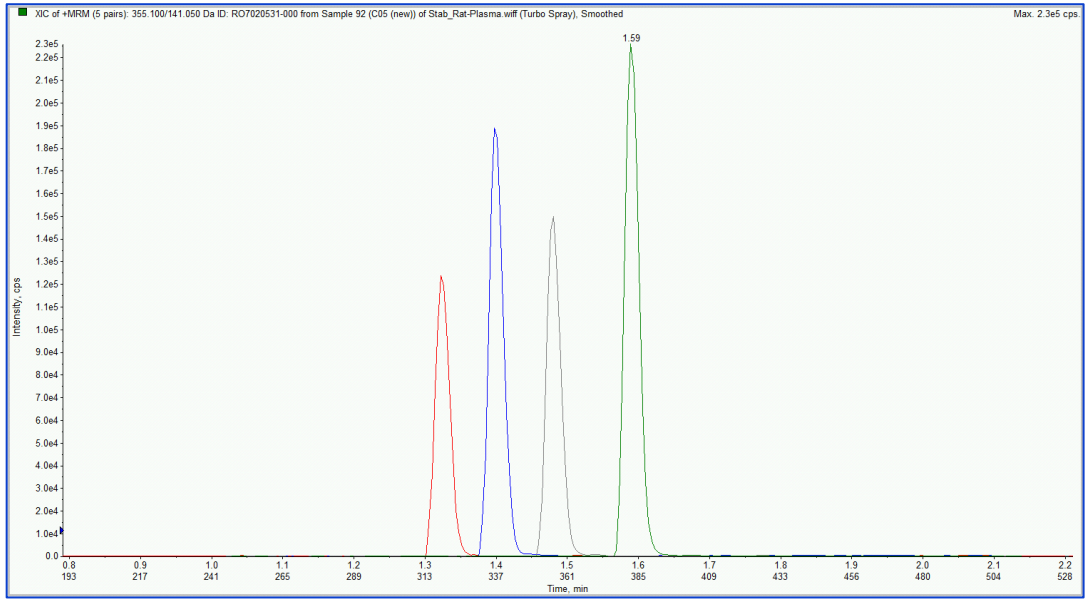
Adapted gradient, still good retention factor (k') but much better chromatographic selectivity
Column dimensions: 2.1x50mm, 1.7µm, RP

- Impact on already measured study samples luckily negligible
- Extra time and effort to adapt and revalidate the method

Case example 2: Timelines and critical materials for the method

Multiplexed method for parent compound plus 3 metabolites

- LCMS method for a prodrug compound plus 3 of its metabolites developed and qualified internally
- Chromatography considered critical – Baseline separation between all compounds to ensure specificity

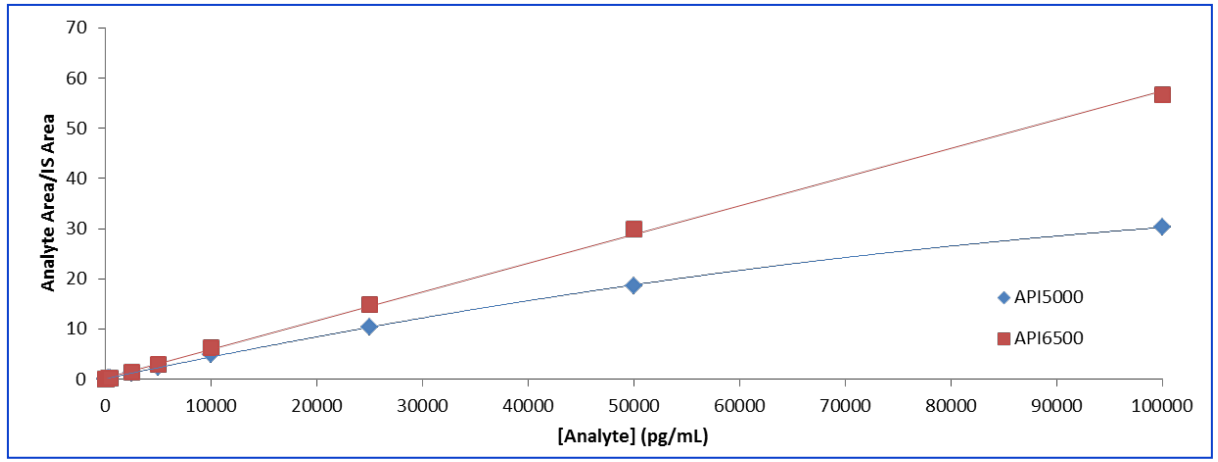


➤ Method ready to be transferred to external partner

Case example 2: Timelines and critical materials for the method

Multiplexed method for parent compound plus 3 metabolites

- Analytical column ordered too late by external partner due to missing exact column information
- Another column used, adapted gradient to ensure baseline separation between the different compounds
- Linearity turned out to become an issue with the adapted method – Loss of linearity at higher concentrations



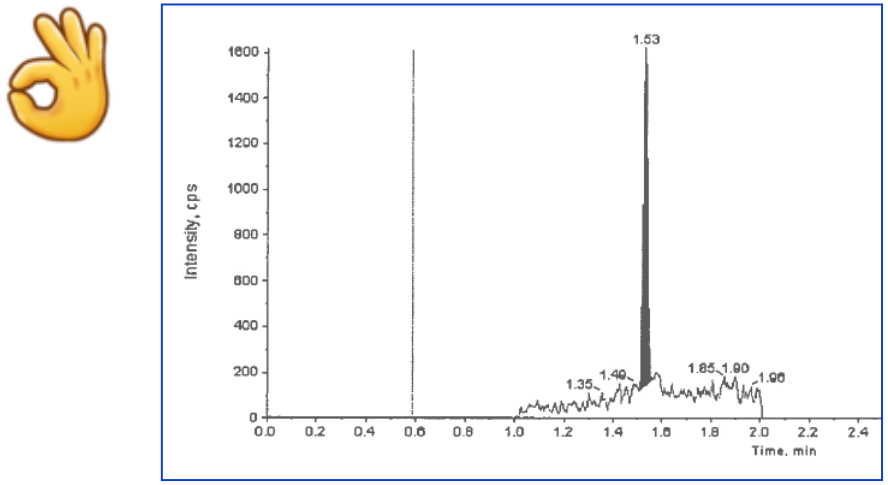
- No time to investigate
- MS used in the method was switched from an API5000 to an API6500, solving the linearity issue
- Method could be successfully validated

- Critical materials for a method should always be ordered asap
- Importance of timely communication between both bioanalytical sites

Case example 3: Change of intended use – Target sensitivity

Small molecule compound in Ophthalmology Project

- Some methods need high sensitivities to fully elucidate the PKs (quantification at late time points)
- In that case the methods are developed and qualified internally, making sure the required sensitivity can be reached
- Example here: Method with an LLOQ of 10 pg/mL, based on LLE, using uHPLC chromatography and sensitive MS detection

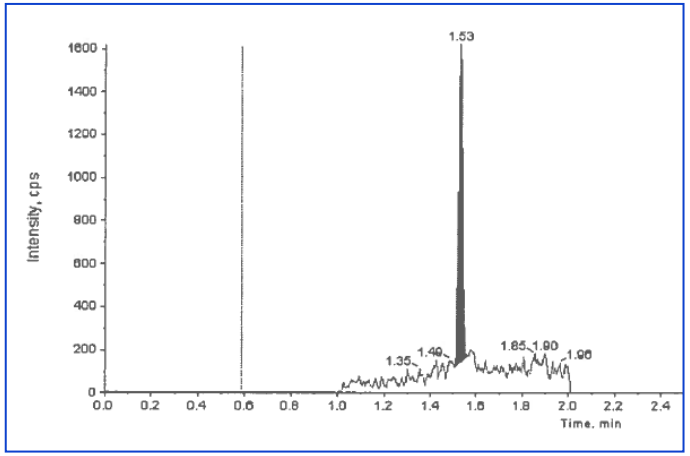


- Robust LLOQ
- Method ready to be transferred to external partner for support of GLP TOX studies with explicit requirement of having same LLOQ

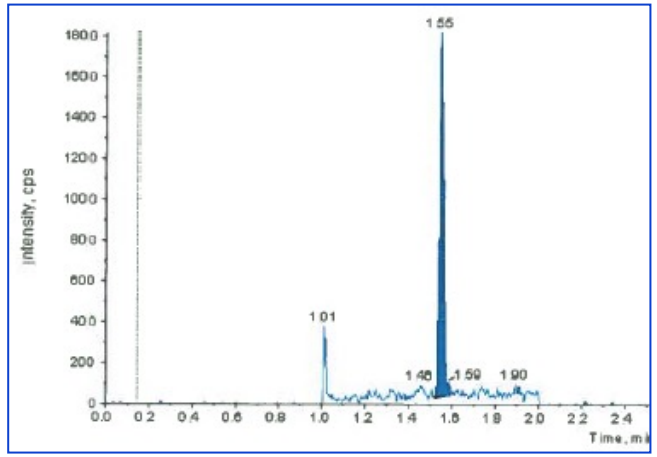
Case example 3: Change of intended use – Target sensitivity

Small molecule compound in Ophthalmology Project

- Project team redesigned the TK studies, original method developed for PK studies was way too sensitive
- Method had to be adjusted: Calibration range was adapted, to avoid performing dilutions into validated range



Initial LLOQ – 10 pg/mL



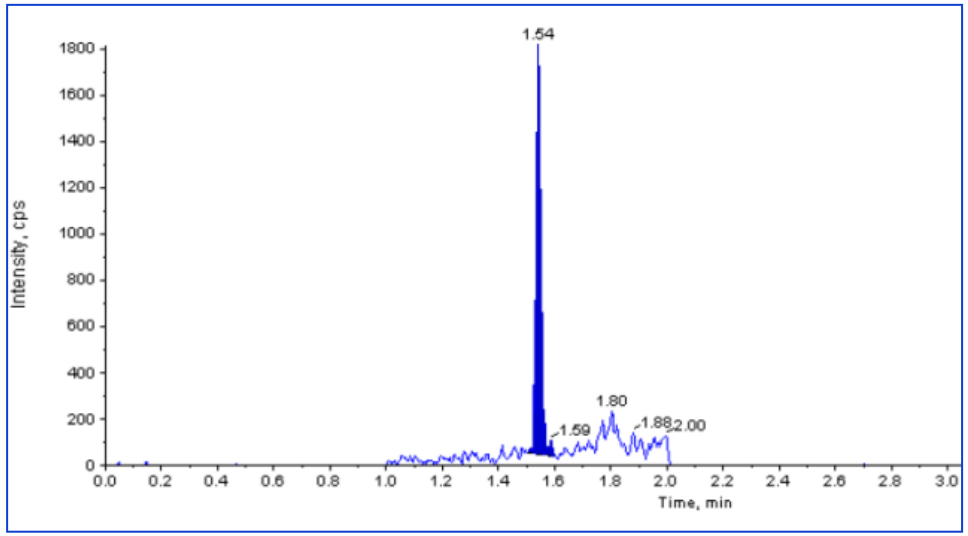
New LLOQ – 100 pg/mL
High range method – 10 fold higher range – Still same dynamic range of detector

- Method adapted successfully
- Much easier, less sensitive method could have been developed and validated upfront, avoiding time losses
- Shows importance of timely communication within project teams

Case example 4: Equipment comparability at both sites

Small molecule compound in Ophthalmology Project

- LCMS method for a small molecule lipophilic compound, developed and qualified in house to support FiH clinical study
- Sensitive method required, LLOQ of 10 pg/mL

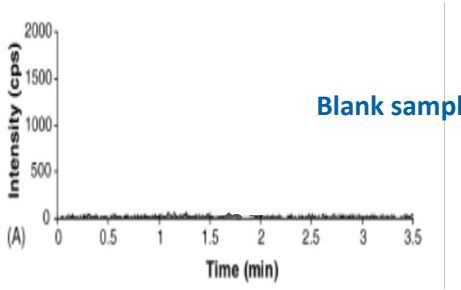


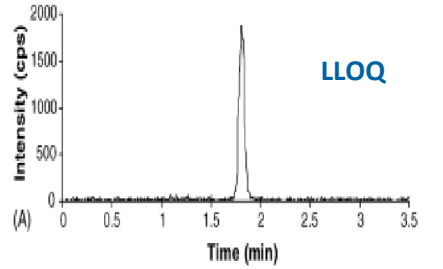
➢ Method ready to be transferred to external partner

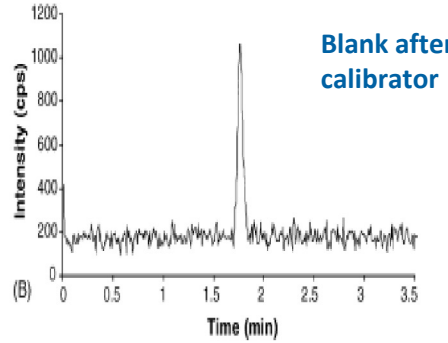
Case example 4: Equipment comparability at both sites

Small molecule compound in Ophthalmology Project

- Method performance at external lab was good, except chromatographic carry-over which was too high
- Exactly same LCMS systems used at both sites – Carry-over type?







- ➢ Carry-over found could be identified as Autosampler carry-over
- ➢ Difference in **Firmwares installed** on the LC systems was leading to Autosampler Wash programs not being identical
- ➢ Shows importance of details, which can affect drastically method performances

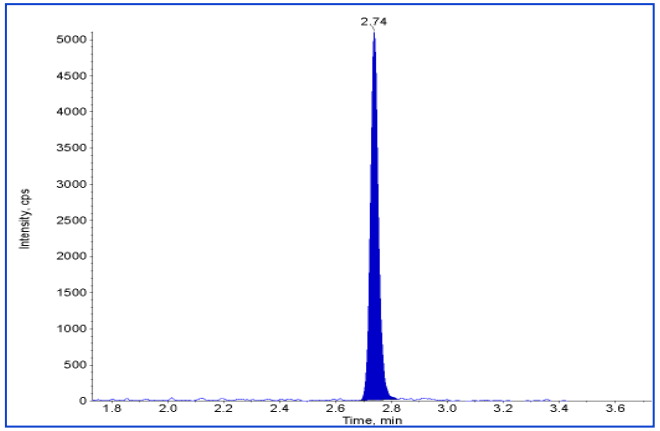
Case example 5: Equipment comparability at both sites

Lipophilic compound with challenging chromatography

- LCMS method for a small molecule lipophilic compound, developed and qualified in house
  - ➢ Chromatography was nicely tailored for the compound

Time (min)	A (%)	B (%)	Flow (mL/min)
0.00	70.0	30.0	1.00
0.40	70.0	30.0	1.00
2.90	50.0	50.0	1.00
3.00	5.0	95.0	1.00
4.00	5.0	95.0	1.00
4.10	70.0	30.0	1.00
4.50	70.0	30.0	1.00

Column: core-shell, PFP, 110A, 2.6µm, 2.1 x 50mm



- ➢ Method ready to be transferred to external partner

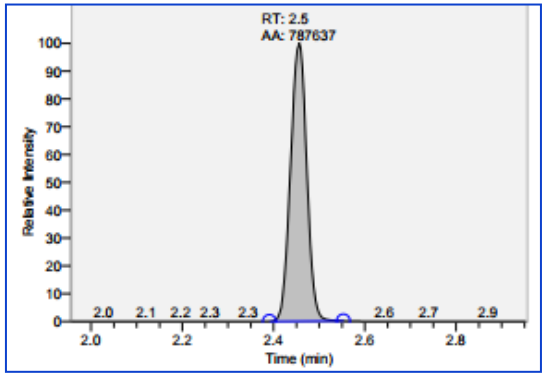
Case example 5: Equipment comparability at both sites

Lipophilic compound with challenging chromatography

- Observed back-pressure with the initial method was too high for the external partner
  - ➢ Chromatography had to be adjusted trying to keep a good retention factor and chromatographic resolution

Time (min)	A (%)	B (%)	Flow (mL/min)
0.00	70.0	30.0	0.650
0.40	70.0	30.0	0.650
3.00	30.0	70.0	0.650
3.10	5.0	95.0	0.650
4.00	5.0	95.0	0.650
4.10	70.0	30.0	0.650

Column: core-shell, PFP, 110A, 2.6µm, 2.1 x 50mm



➢ Method adapted and validated successfully

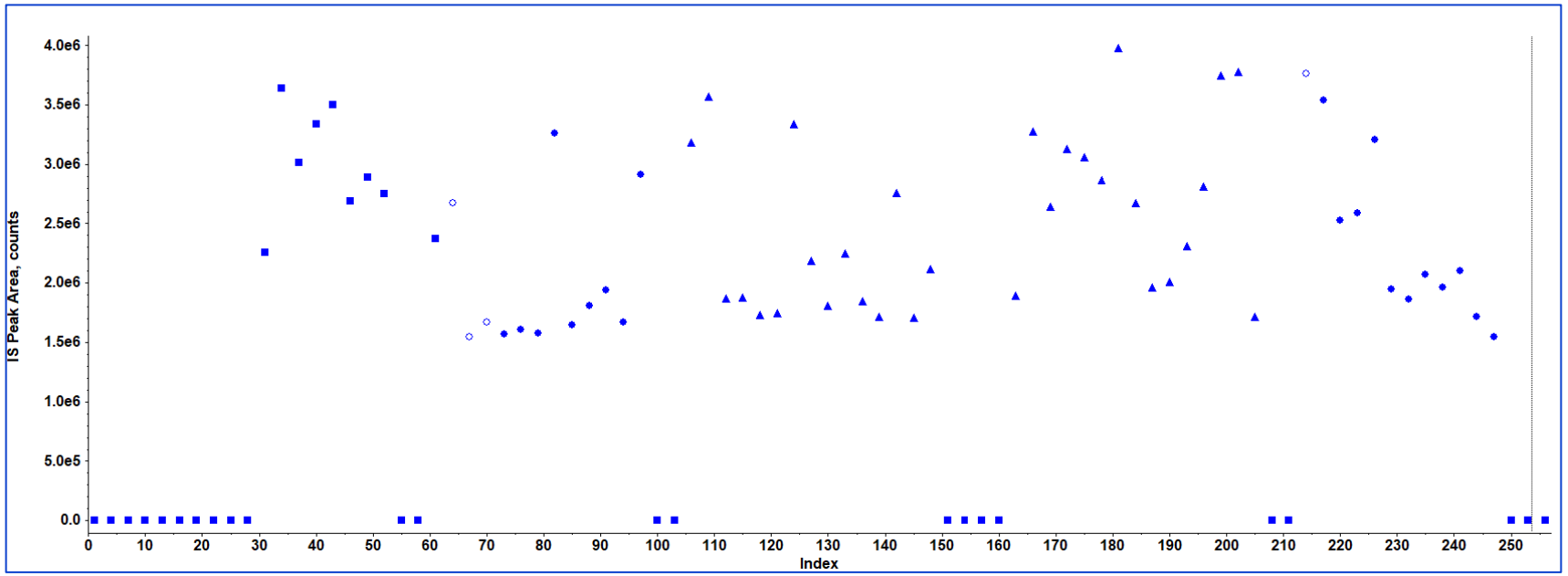
➢ Method could have been developed accordingly up front, avoiding unnecessary adjustments

➢ Shows importance of equipment comparability, to be able to apply same parameters at both sites

Case example 6: Lack of information, details about the method

Oligonucleotide method using an anion exchange SPE

- Oligonucleotide method transferred internally from one group to another – Extraction based on tailored anion exchange SPE
- First test run showed following SIL-IS trend:

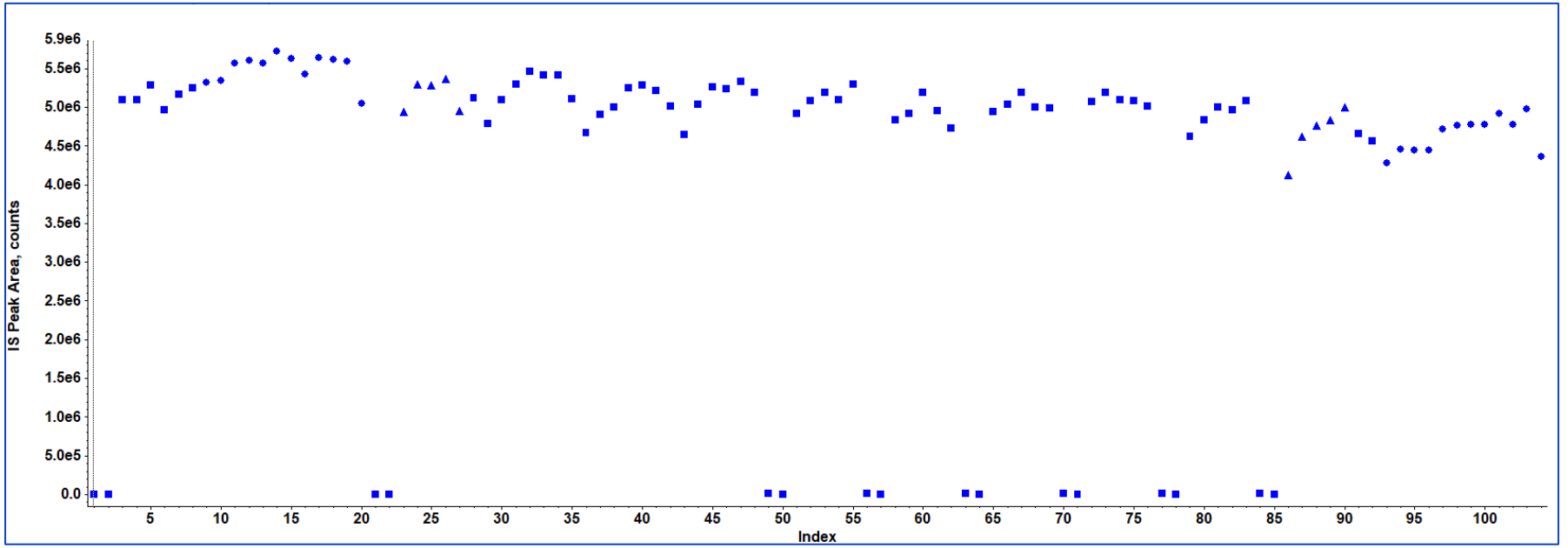


➢ Really high SIL-IS response factor variations – Relative response between analyte and SIL-IS was stable

Case example 6: Lack of information, details about the method

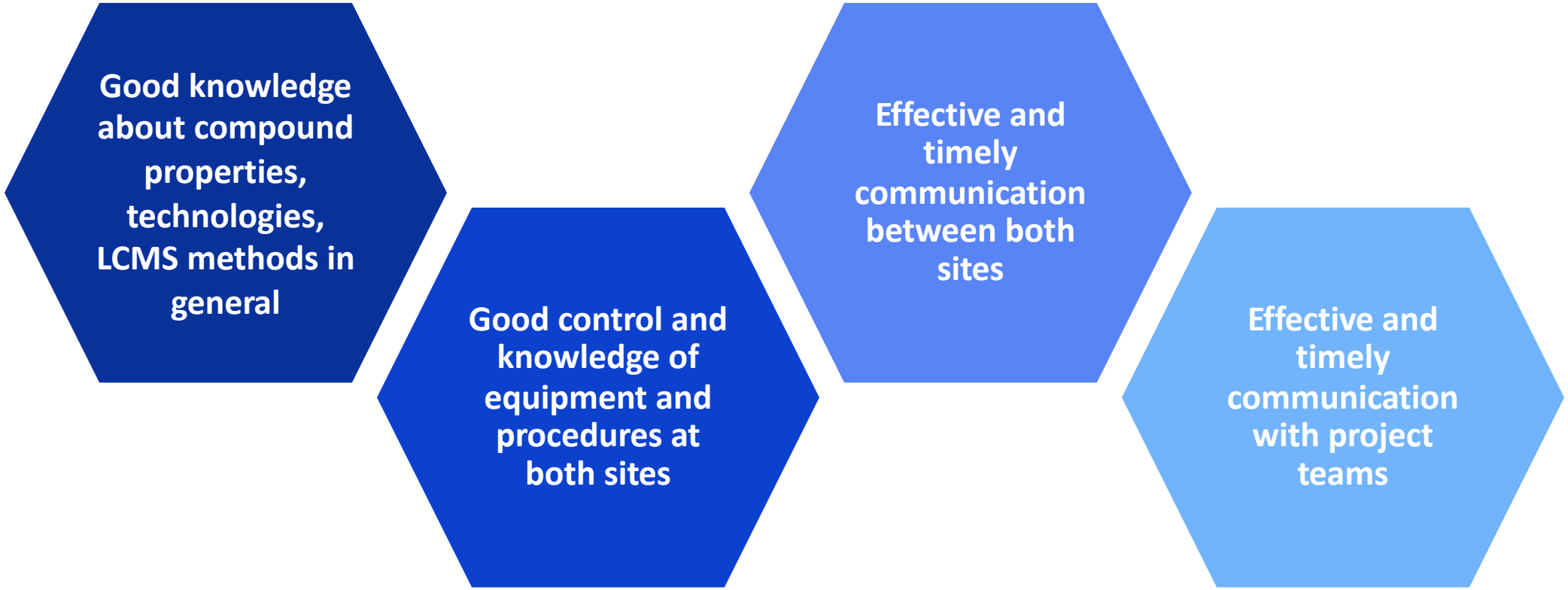
Oligonucleotide method using an anion exchange SPE

- After investigations, the SPE extraction turned out to be the root cause for the SIL-IS variations
  - Issue could be solved by centrifuging samples prior to loading onto the SPE and not loading entire sample



- Much better SIL-IS response factor variation – Method ready for sample measurement
- Despite a well established protocol, small variations can make a big difference

Key learnings and outlook



- **Outlook:** What about **outsourcing of large molecule LCMS methods?** Can be even more challenging (methods at edge of sensitivity, usage of more critical reagents, materials, more complex sample processing procedures)....

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Thank you for your attention

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Doing now what patients need next