ULTRAFAST LC-UV-ELSD-MS CONFIRMING THE HIGH QUALITY OF INVENTIVA'S SCREENING COLLECTION

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Frédéric Bell [1], Clément Gaiffe [1], Amandine Vessot [1], Jérôme Amaudrut[2], Sylvaine Marie [3], Philippe Masson [3],

[1] Analytical Sciences Group, Medicinal Chemistry, [2] Computer-aided Drug Discovery, Medicinal Chemistry, [3] Compound Management Team, Biology & Screening, **INVENTIVA** 50 rue de Dijon, Daix. 21121- France

OVERVIEW

INVENTIVA is a fully integrated Drug Discovery company based in Dijon (France) dedicated to the finding of novel drug development candidates.
The **Analytical Sciences Group** (7 Scientists and Technical Experts) is providing an extensive panel of techniques encompassing 1D & 2D NMR, heteronuclear NMR, quantitative NMR, UPLC-UV-ELSD-MS, GC-MS, LC-HRMS, Preparative LCMS and services like solubility or lipophilicity determination.
The **Compound Management Team** (4 Scientists and Technical Experts) is **in charge** of the whole INVENTIVA screening collection in both solids and ready-to-use DMSO solutions **formats**.

Our library is housed in a state-of the-art facility. Powders are stored in standardized containers in an automated **carrousel** (Kardex). Liquids are stored in microtubes under inert atmosphere at low temperature (Microtube Store, Hamilton).
All the samples are managed via Mosaic **software** (Titian) and are securely tracked throughout the process via barcodes. INVENTIVA is constantly improving the collection by adding new compounds in order to maintain novelty and diversity.
This posters describes the 2014 **IVAQual** campaign that assessed the quality of the INVENTIVA **s**creening compound collection by ultrafast liquid chromatography hyphenated to a triple detection system UV-ELSD-Mass Spectrometry over a random subset of 20 000 compounds.,

INTRODUCTION

IVALib is INVENTIVA**'s** proprietary library of ~240,000 compounds which has so far never been made available to outside parties. The library was designed, over the years, by our medicinal chemists and **modelers** with drug discovery programs and high throughput screening in mind. Overall diversity is excellent (1) and 2/3rd of the library is original compared to ZINC Everything (http://zinc.docking.org). 100% of the library is stored as frozen DMSO solutions and is ready to be screened and 73% is also available as solid samples.

As of today**, various target families** have been successfully screened (GPCR's, nuclear receptors, kinases, enzymes, protein-protein interactions, epigenetic targets) with multiple hit series progressed to lead series and more than 5 clinical candidates.

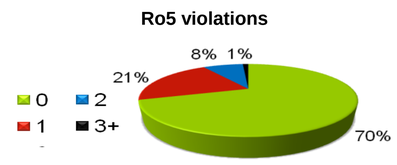


Fig 1 : quality of the IVALIB collection represented by the number of violations to the Lipinski rule-of-five (Ro5).

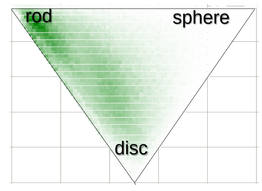


Fig 2 : density of IVALIB compound population In a 3D shape representation

The quality of the compound collection has a great influence on the success of biological screening(2). It is nowadays well recognized that if the success of a HTS largely relies upon the quality of the biological screen, it also dramatically depends on the size and the chemical diversity of the collection, as well as the chemical and physical quality of **the** compounds **screened.**
At INVENTIVA all the compounds delivered to the Compound Management are checked by NMR and LC-MS with a purity criteria ≥ 90% by UV in UPLC/UV/MS. Over time **however** the purity might have deteriorated **in particular as a consequence of reformatting.** (3). Precipitation may also have occured, mainly due to water adsorption by the DMSO, especially during the frozen-thaw cycles. This is likely to drastically affect both the biological results and the quality control checks .
In order to assess the current quality of our collection, we decided in 2014 to setup an extensive quality control project named **IVAQual** which consisted of the UPLC-UV-ELSD-MS analysis (4) of more than 20 000 randomly selected compounds.
The **IVAQual** project was organized in five different stages : the design & preparation stage, the technical pilot stage (1 000 compounds) followed by the operational pilot stage (5 000 compounds), the production stage (14 000 compounds) and finally the data analysis **and** reporting stage.
The **preparation** mainly consisted in establishing the list of 20 000 compounds representing the vast diversity of the whole collection of 2 **43**0 kcpds, immediately followed by plate preparation The compounds were delivered in 384w plate, each well containing 30 µL of 1 mM DMSO solutions.

METHODS

IVAQual was carefully designed to establish a robust and efficient process supported by a clear decision tree. Compound Management, Medicinal Chemists and Analysts were all involved during this essential phase.

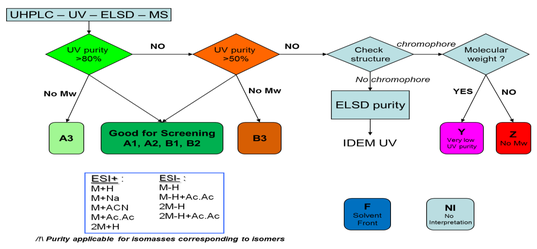


Fig 3 : Decision tree

UPLC-UV-ELSD-MS operational parameters were set up and optimized during the "technical pilot" performed with 960 diverse compounds. Run time was set to 150 sec of which 123 sec are fully analytically exploitable.

Column / Stationary phase	ACQUITY UPLC BEH C18 50x2.1 mm 1.7 µm
Column temperature	Thermostat : 45°C
Mobile phase	Water/Acetonitrile (+0.1% Acetic Acid)
Elution mode	Gradient 2.5min
Pressure range	5 000 - 10 000 PSI
Flowrate	0.8mL/min

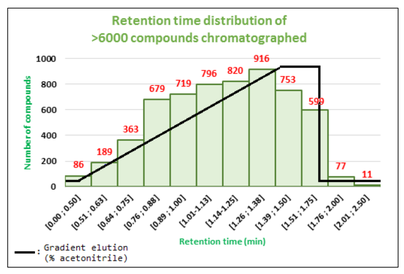
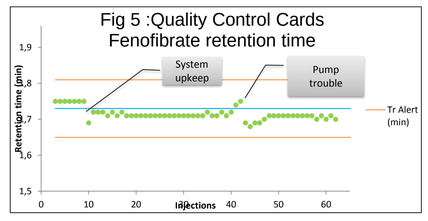


Fig 4 : Distribution of the 6 000 compounds set by retention time all along the chromatographic run, showing a well-distributed shape and demonstrating the suitability of the chromatographic gradient.

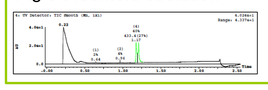
It is absolutely mandatory to secure the quality and the stability of the process all along the duration of **the project. To this effect,** a reference solution was injected everyday and Quality Control Cards were filled in with relevant data (retention time tr, theoretical plates N), in order to detect as early as possible any trouble like ageing of the chromatographic column or instrument weaknesses.



RESULTS

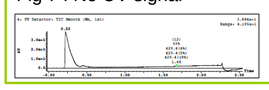
The organization of the project was set up in a way that the analyst in charge could review every chromatograms and chemical structures in order to ensure that no false positive or false negative may occur.
It was anticipated that a minority of cases would require in depth analysis and could eventually be subject to individual interpretation. To ensure that no operator **bias** was introduced, a set of 63 complex cases was submitted independently to the analyst in charge of **IVAQual** and **to** two senior analysts. It resulted that less than 5% of these difficult cases were subject to slight interpretation**al differences,** representing less than 1% of the **IVAQual** set.
Examples of problematic data requiring manual interpretation :

Fig 6 **:** Isomers coelution



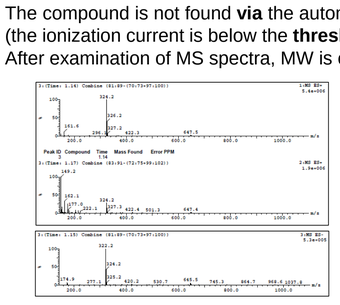
Z/E or Cis/trans isomers are very likely to occur with respect to the chemical structure hence both peaks can be gathered The class of the compound changes from Y to A1

Fig 7 : No UV signal



The very weak UV signal leads to an underestimated purity, then after careful reprocessing, the class of the compound changes from Y to B1

Fig 8 : Expected compound not found by system



The compound is not found **via** the automatic process (the ionization current is below the **threshold**).
After examination of MS spectra, MW is confirmed

After correction the status of the compound changes from A3 to A1

As third step, the operational pilot was successfully performed and led to a fine tuning of the operational parameters,
Finally, the production stage was initiated on schedule, with a throughput of 1000 samples/week.

A very limited number of maintenance shutdown or instrument failures allowed us to complete the production stage 6 weeks ahead of the deadline.

Good design and planning facilitated data interpretation and reporting was almost immediate.

A very high quality of our screening collection was demonstrated, based upon this representative subset of 20 000 compounds (~9% of the total screening collection),

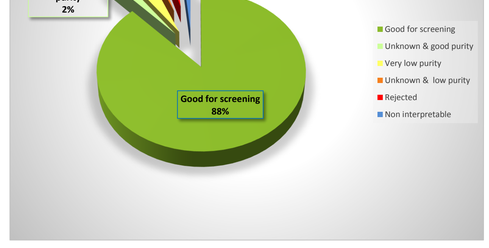


Fig 9 : Distribution of the classes of compounds at the end of the project

CONCLUSIONS

IVALIB is INVENTIVA's 243 000 mature compound screening collection, **IVAQual** is an ongoing IVALIB quality control **process**. Our 2014 I**VAQual** campaign has clearly confirmed the high quality of **IVALIB**'s ready-to-screen in solution set. 88% of the randomly selected representative set of 21 072 compounds are unambiguously confirmed by UPLC-UV-ELSD-MS technique. Furthermore, 73% **of the compounds** are available as solid samples and freshly prepared solutions will **replenish** the collection.
Careful planning **and a clear** decision tree enable**d** us to run an **IVAQual** campaign over a 4 month period only and **without impacting the analytical department regular activity.**

References & contacts

(1) Poster exhibited at EuroCUPVII, Méry-sur-Oise, France, May 14-16, 2014, http://lanyrd.com/2014/eurocup7/
(2) Lane, S-J**.** ***et al.*** **2006**. Defining and maintaining a high quality screening collection : the GSK experience. *Drug Discovery Today*, 11 (5/6), 267-272.
(3) Bowes, S *et al*. **2006**. Quality assessment and analysis of Biogen Idec Compound Library*, Journal of Biomolecular Screening*, 11 (7), 828-835.
(4) High throughput chemical integrity assessment using Waters UPLC open architecture system, *Waters application note*, Marian Twohig

Contacts :
frederic.bell@inventivapharma.com
amandine.vessot@inventivapharma,com
philippe.masson@inventivapharma.com