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# Proprietary Compounds Screener Database: A powerful tool for the identification of organic compounds during controlled extraction studies

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## SUMMARY

Nelson Labs Europe has developed a Compounds Screener Database containing over 2000 Volatile, Semi-Volatile and Non-Volatile Organic Compounds which allows performing a high level of first pass extractable identification in Controlled Extraction Studies. The compound identification in the Nelson Labs Europe Screener Database is based on both the confirmation of the retention time of the compound and on its mass spectrum, using hyphenated chromatographic techniques (Headspace-GC/MS, GC/MS and LC/MS). The Screener Database is not only built from commercially available standards, it also contains synthesized compounds or compounds which were isolated from a material extract. In addition, the "Screener Database" identifies *Most Probable Compounds (M.P.C.)* and *Tentatively Identified Compounds (T.I.C.).* These M.P.C. and T.I.C. are compounds – often encountered in Controlled Extraction Studies – where no analytical standards are available but where additional structural information can be given. Further developments of the Screener Database will focus on 2 areas: (1) increase the number of compounds – identified in Controlled Extraction Studies – of which the analytical standards are commercially available and (2) perform further identifications of unknown extractables, using advanced analytical "accurate mass" techniques such as GC-ToF-MS, LC-Q-ToF-MS and FT-MS. The identified compounds will subsequently be included into the Compounds Screener Database. This ongoing development of the Compounds Screener Database will further increase the level of identifications on a first pass basis in future Controlled Extraction Studies, performed on materials used in the manufacture of Pharmaceutical Containers.

## INTRODUCTION

The importance of Extractable / Leachable testing for Container Closure Systems (CCS) in the Pharmaceutical Industry has grown considerably in the last few years, driven by an increase in global regulatory requirements as well as by well-documented incidents where impurities in the contained drug product – introduced by the Container Closure System via leaching – were found to be harmful to the patients. This has lead to a higher level of testing of Containers / Closures and an increased level of documentation of

the selected C/C in a submission dossier. An initial step in the E/L evaluation process is to perform Extractable Studies on the Containers / Closures, this to learn as much as possible about the extractables profile of the materials, used in the manufacture of Pharmaceutical containers. Ideally, all compounds, detected in Controlled Extraction Studies, should be fully identified and characterized. This allows Toxicologists to perform an in depth evaluation of the material, based upon the actual toxicity data of the compound or – if no toxicological data are available – via a "Structure Activity Relationship" (SAR) evaluation. Currently, the identification of extractables is typically based upon the mass spectral fit with commercial MS-databases (such as NIST or WILEY). Although these commercial databases contain a large amount of mass spectra (250.000 or more), these databases are not always optimized for material extractable research. This means that a lot of mass spectra of compounds, commonly found in material research, will not be present in these databases. In addition, a good quality mass spectral fit with the commercial databases does not guarantee a correct identification of the compound. As a consequence, the outcome of such a first pass Controlled Extraction Study does not always allow a full identification of all compounds in the extractables profile of a material. In order to guarantee a full identification of the extractables profile, further efforts need to be made in second pass extractables testing. These additional efforts can range from spiking experiments *(where the identity of the compound is confirmed by spiking the extract and to confirm its retention time and mass spectrum)* to a high level of analytical testing, such as NMR, GC-NMR, GC-FTIR, GC-ToF-MS, LC-ToF-MS,… It is evident that, if the level of identification in first pass experiments could be increased substantially *(e.g. by the development of a "Screener Database")*, the efforts for further identifications in second pass experiments could be reduced substantially. As a consequence, pharmaceutical companies would need to spend less on internal analytical resources for further identification of unknown extractables.

## THE PROPRIETARY COMPOUNDS SCREENER DATABASE: HOW DOES IT WORK?

The instruments, used in the database development, are "Standard" Agilent Analytical Instruments (Headspace GC/MS: Agilent G1888 Headspace Sampler, coupled to an Agilent 6890N GC with Agilent 5975B Inert Mass Spectrometric Detector; GC/MS; Agilent 6890N GC System, Agilent 5975B Inert Mass Spectrometric Detector; LC/MS: LC: Agilent 1100, MS: Agilent 1100 Series LC/MSD Trap SL). These types of hyphenated chromatographic techniques are broadly used across different labs for the first pass identification and (semi)-quantification of extractables in Controlled Extraction Studies. By performing the first pass extractable testing on "standard" instruments, the project cost for a first pass extractable study can be kept at an acceptable level.

The extractable identification database contains a very broad group of organic compounds with diverse chemical structures, since these compounds (e.g. Anti-Oxidants, Nucleating Agents, UV-stabilizers, Slip Agents, Plasticizers, Pigments, Vulcanizers, Initiators, Accelerators, Solvent residues, Residual Monomers, Oligomers, Glue/Adhesive residues, Polymer (Additive) Degradation Compounds…), can originate from a broad range of materials (e.g. LDPE, HDPE, PP, Rubbers (Butyl, Chlorobutyl, Bromobutyl, EPDM, Isoprene…), PVC, Multi-layer Films, COC, COP, PBT, Polyacetal (POM), ABS, Silicone, Polycarbonate, PET, PETG, Glue/Adhesives, Nylon,…). Examples of typical compounds in the database can be found in Figure 1. Therefore, a crucial step in the development of

the Screener database – based upon both retention time and mass spectrum match – was to optimize and define the right set of chromatographic conditions for the three techniques used in the database (Headspace GC/MS (volatile organic compounds), GC/MS (semi-volatile organic compounds) and LC/MS (non-volatile organic compounds)). In addition, the chromatographic methods for Headspace GC/MS and GC/MS are run in "retention time lock" mode, this to reduce the retention time shift during sample analysis.

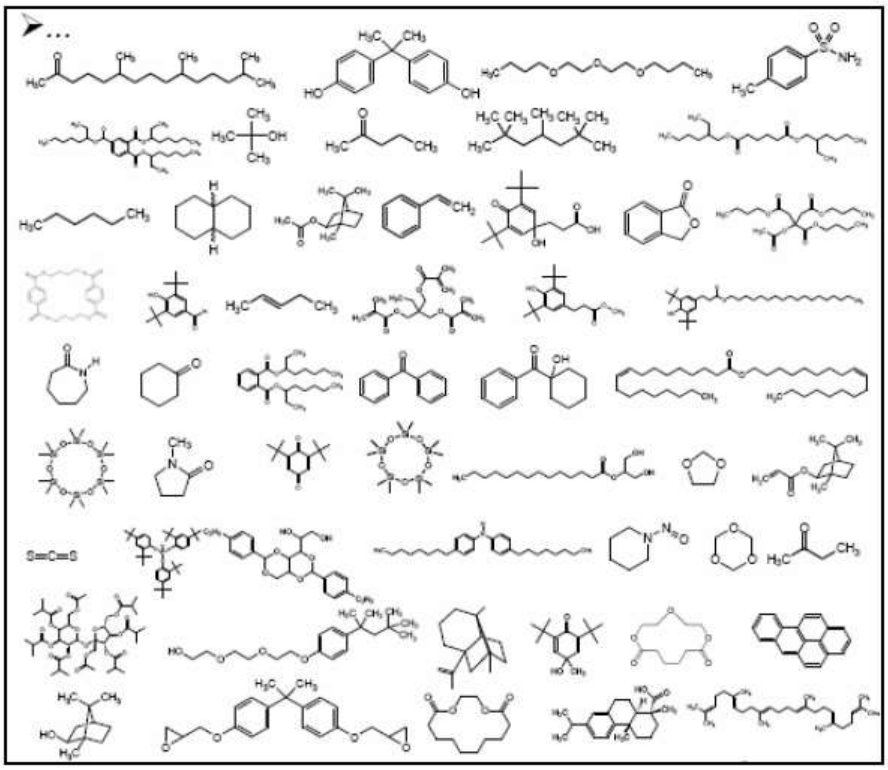


*Figure 1: example of typical compounds, included as I.C. in the Compounds Screener Database*

The list of selected compounds included into the Screener Database was mainly based upon the identification results of Controlled Extraction Studies, carried out by Nelson Labs Europe over the last 10 years. The laboratory has had a broad exposure to different types of polymeric materials and rubbers, sterilization modes, and process treatments etc… The identification results of polymeric/rubber extractables, found in over 2000 projects, were bundled and prioritized, based upon importance and frequency of occurrence. In addition, compounds referred to in literature or compounds, presented in case studies at E/L-conferences were also included. Finally, regulatory guidelines *(such as directive for the plastic materials and articles intended to come into contact with food, 2008/39/EC)* also contributed to the list of selected compounds.

For each selected compound it was verified if an analytical standard was commercially available. In this case, an analytical standard was purchased from a qualified vendor. Important herein is that most standards were supplied with a Certificate of Analysis, this to prove and document the identity and the purity of the compound. In a next step, an analytical working standard was prepared from each individual compound in a suitable solvent at a concentration level which allows obtaining high quality mass spectra (e.g. 100 mg/L). The analytical working standard was then analyzed under standard chromatographic conditions using the optimized Nelson Labs Europe screening methods. Subsequently, the retention time for the compound was determined and the mass spectrum of the compound was recorded. In the evaluation of the mass spectrum of the compound, obtained via Headspace GC/MS or GC/MS, 4 ions are selected based upon their sensitivity or specificity for the compound. One of these ions is the "target ion", the other three ions are "qualifier ions".

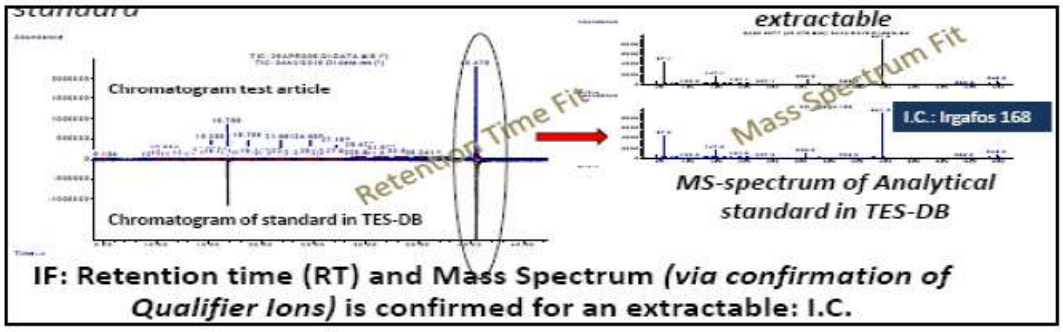
When identifying an extractable using the Compounds Screener Database via GC/MS, a first step in the process is to verify for a retention time match with one of the compounds in the Database. When the retention time of the compound, found in a Controlled Extraction Study corresponds to the retention time of a compound in the Compounds Screener Database, the target ion and the three qualifier ions of the compound are compared to the target ion and qualifier ions of the compound in the Screener Database. Not only should these 4 ions be present, they should also be present at certain predefined ratios. When this condition is fulfilled, the compound was uniquely identified by the Screener Database.

The GC-MS Nelson Labs Europe Compounds Screener Database defines 4 classes of identification:

### 1. I.C.: Identified Compound

For I.C.'s, a Certified Standard is present at Nelson Labs Europe. The preparation of the analytical working standard, as well as the analytical results of the chromatographic analysis and mass spectral results of the analytical standard, is fully documented/traceable at Nelson Labs Europe. A Schematic approach of the identification of an I.C. is given in Figure 2. The Proprietary Compounds Screener Database contains approximately 1.700 I.C.'s across the three different techniques *(Headspace GC/MS (volatiles), GC/MS (semi-volatiles) and LC/MS (non-volatiles))*.

*Figure2: Schematic overview of the identification steps for an Identified Compound (I.C.)*




## 2. M.P.C.: Most Probable Compound

For M.P.C.'s, an Analytical Standard is not commercially available. However, there is an excellent fit with MS-library (PBM>80% or RMF>800), with MS-data in Scientific Literature or with available MS-data (after synthesis/isolation in other laboratories. The Chromatographic/Mass Spectral results of each M.P.C. are fully documented/traceable at Nelson Labs Europe. A Schematic approach of the identification of an M.P.C. is given in Figure 3. The Compounds Screener Database contains approximately 150 M.P.C.'s across the three different techniques *(Headspace GC/MS (volatiles), GC/MS (semi-volatiles) and LC/MS (non-volatiles))*.

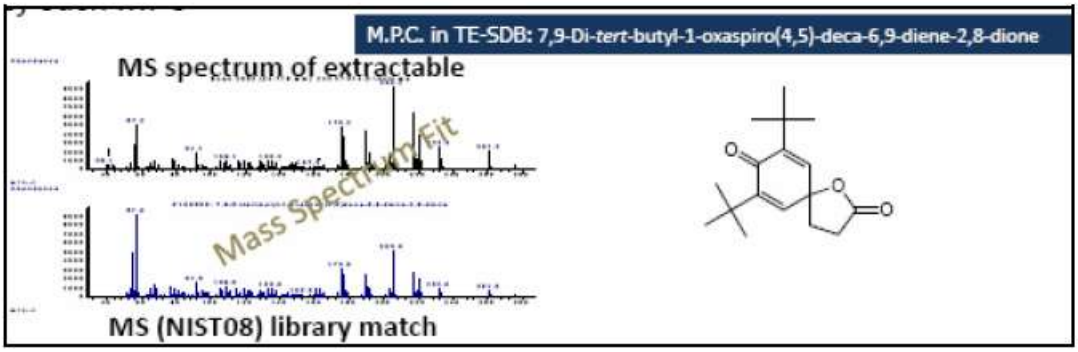



*Figure 3: Schematic overview of the identification steps for a Most Probable Compound (M.P.C.)*

### 3. T.I.C.: Tentatively Identified Compound

Also for T.I.C.'s, the Analytical Standard is not commercially available. In addition, the fit with the commercial MS libraries (e.g. NIST, WILEY) gives a lower quality fit (50%-80%). For these compounds, only limited structural information can be provided. The Compounds Screener Database contains approximately 150 T.I.C.'s across the three different techniques *(Headspace GC/MS (volatiles), GC/MS (semi-volatiles) and LC/MS (non-volatiles))*.

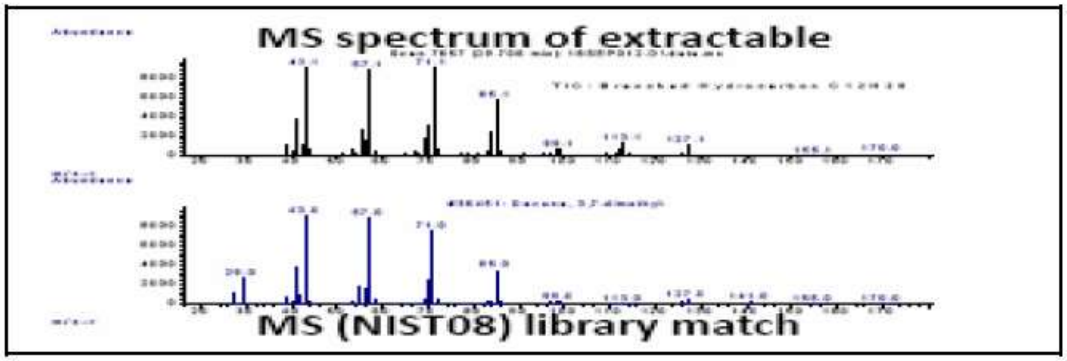



*Figure 3: Schematic overview of the identification steps for a Tentatively Identified Compound (T.I.C.)*

### 4. U: Unknown Compounds

For Unknown compounds no Analytical Standard is commercially available and there is no fit with commercially available MS-libraries. As a consequence, no structural information can be given in first pass experiments. The Compounds Screener Database contains approximately 50 U.'s across the three different techniques. The reason to include frequently found Unknowns into the Database is to internally streamline the information on the knowledge of the unknown compound. Once further attempts to identify the compound have been made, the identification status of the compound can then be easily upgraded from U to T.I.C. or M.P.C.

The identification process of the Screener Database for LC/MS is similar to the identification process of GC/MS, described above. However, future work in this field will further optimize and automate the evaluation process.

TES-DB: BENEFITS

The benefits of being able to use the Compounds Screener Database, using a double confirmation (RT and MS) for first pass Controlled Extraction Studies, are numerous. It is evident that the unique identifications, provided by the Compounds Screener

Database, avoid any concerns about the identity of the compound if it is present in the Database. Indeed, the use of the Database provides high quality identifications in a first pass testing. The way the Nelson Labs Europe Compounds Screener Database is set-up also largely avoids the need for second pass spiking experiments to confirm the identity of a compound via retention time verification. It is evident that the efforts of further identifications in second pass experiments could be reduced dramatically if the level of identification in first pass experiments could be increased substantially through the use and further development of a Screener Database,. As a consequence, pharmaceutical companies need to spend less on (expensive) internal analytical resources for further identification of unknown extractables.

For an analytical lab which performs E/L studies on a daily basis, the introduction of the Compounds Screener Database streamlines the level of identification of extractables across the organization. In a larger organization, the level and quality of mass spectral interpretation can be affected at various levels (analysts, system owner, lab supervisor, study directors). Depending upon the skills and knowledge of mass spectral interpretation of each individual, the identification could come out differently. By using the Compounds Screener Database, less manual MS-interpretation is necessary which leads to a better harmonization in – and a consistently higher level of – identifications in Controlled Extraction Studies. In addition, by making the interpretations less operator dependent, the results of a Controlled Extraction Study – for a certain material – can be compared much easier across different testing labs, using a similar identification approach.

## FUTURE WORK

It is evident that theNelson Labs Europe Compounds Screener Database is a growing Database, where new compounds will be added on an ongoing basis.

An initial focus of the future Compounds Screener Database development programme will be to further increase the number of “Identified Compounds” in the Database. This means that “new” extractables, encountered during the ongoing and future E/L work atNelson Labs Europe, will be purchased (when commercially available), working standards will be prepared and analyzed and the retention time and Mass spectrum will be added to the Database. However, for a large group of extractables which are often encountered in Controlled Extraction Studies (as M.P.C. or T.I.C.) analytical standards are not commercially available. For these compounds, attempts will be made to synthesize these compounds or to isolate the compounds from the extracts (via preparative LC with fraction collection) to obtain further structural information if necessary. These synthesized/isolated compounds will then be used as standards to further build the I.C. category.

In addition, Nelson Labs Europe has started up a Research Project (Baekeland Project) to identify unknown compounds, often detected in Controlled Extraction Studies. The first step in the approach will be to analyze the extracts of materials, investigated at Nelson Labs Europe using Accurate Mass GC-ToF-MS. By using Chemical Ionization (CI) GC-ToF-MS, more information can be

obtained about the elemental composition of the molecule. Electron Impact (EI) GC-ToF-MS measurements of the fragments, on the other hand, could lead to more information about substructures or functional groups within the molecule. Combining the results of the accurate mass measurements with results, obtained via other analytical techniques (LC-Q-ToF-MS, FT-MS, NMR), could lead to an increased level of identification (TIC or MPC) for compounds which are currently reported as “unknowns”.