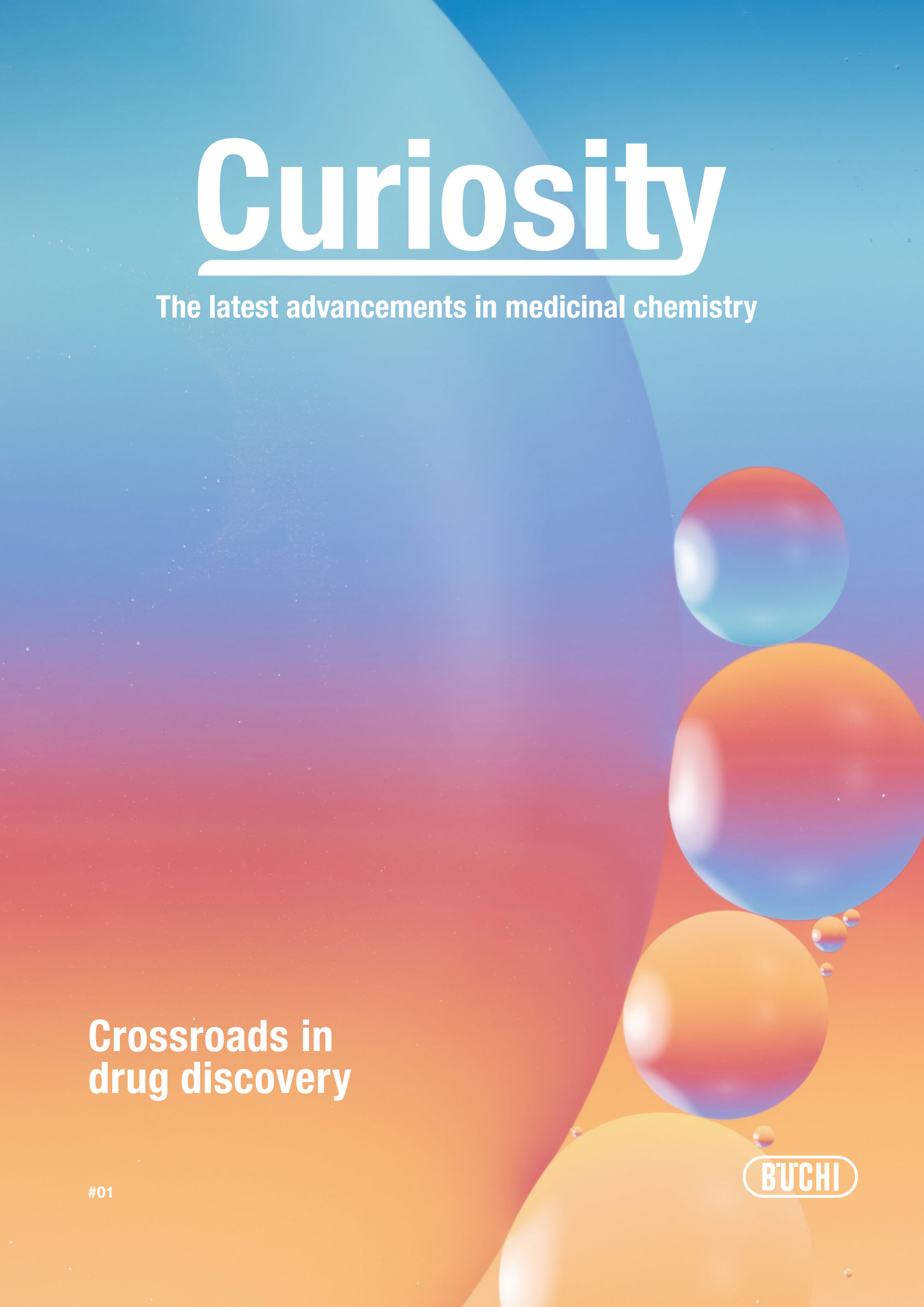
Curiosity

The latest advancements in medicinal chemistry

Crossroads in drug discovery



#01

BÜCHI

From Choice to Informed Decisions

Dr. Ruedi Hartmann
Director Business Unit Pharma & Chemistry
BUCHI Labortechnik AG

Dear reader, it is my pleasure to welcome you to the first edition of “Curiosity Magazine”. We put a lot of thought into covering the most important considerations for those working in pharmaceutical R&D labs. And I am happy to share with you our first issue, dedicated to covering the entire drug discovery and development process with focus on the choices you have available at each step.

Why did we go in this direction? As laboratory technology continues to improve, the capabilities of the instruments grow in equal speed. Users are awarded an increasing number of possibilities to get even more out of their laboratory equipment. But does the gift of choice become an overwhelming burden at some point?

I am here to argue, no. Choice is always a good thing, it is up to you if you want to make use of that choice. But it is your responsibility to inform yourself and make smart decisions. I mean, just because your clothes dryer has functions for anything from towels to ski jackets to dress shirts, does not mean you ever have to use a program outside of quick-cotton-mix. But maybe you should consider it.

Drug discovery and development, is undoubtedly, a lot more complicated than drying clothes and it involves multiple instruments and accessories to help you achieve optimal results. Either way you look at it, the range of methods and lab equipment to help you reach your goal is simply staggering. And for this reason, we have decided to use our first issue of “Curiosity Magazine as a guide through many of the decisions you must make in your drug design workflow.

Just to outline what I mean. At the very start of the drug discovery process, you must already make an essential choice, will you extract or synthesize your compound. If you extract, will you use solvent extraction, supercritical fluid extraction, microwave-assisted extraction or a different method?

As you go on, how will you concentrate your sample in between steps? Will you use a rotary evaporator, a parallel evaporator, a glass oven or even lyophilization? What sizes flasks are available, how many samples can you evaporate at the same time? How safe is your precious sample and does it foam?

Don’t get me started on fraction purification and isolation! Will you use flash LC for pre-purification or directly go to prep HPLC? Is your sample chromophoric or do you need ELS detection or MS? If you are performing chromatography, do you own consumables best suited for your particular application?

Can all of your techniques be easily upscaled for the drug development part of the process?

How are you going to pre-formulate your product? What will your product look like at the end? If you want a powder, will you spray dry, encapsulate, freeze dry and how would that influence the properties of your final product?

My goal is not to make your head explode. Many of you already have established processes in place and are not just starting out. I am here to bring awareness to possibilities that you might be less aware of but might still be better suited to your work. We have really tried to focus on essential bits of information that could help you realize if a certain piece of lab equipment might be exactly what you need for your process.

I mean, the quick-cotton-mix program will get your ski jacket dry, just as a Rotavapor can concentrate your 20 precious samples. But any mean to an end, does not indicate it was the best mean to the best end.

Simply keep an open mind, flip over the next pages, and recognize if the methods and equipment in your drug discovery and development workflow perfectly fit your application. Lab technology is evolving, perhaps it is time that your workflows do too.

Yours,

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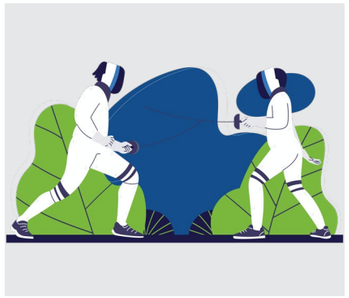
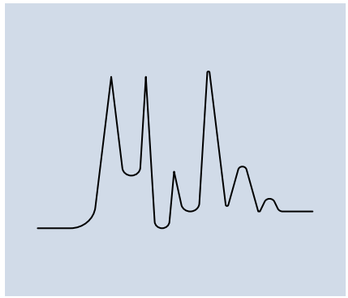


Synthesis vs extraction

Is your lab equipment a deal breaker?

The search for active pharmaceutical ingredient (APIs) and chemical compounds typically begins with a synthesis or an extraction step. The good news: these techniques can be performed on basic laboratory equipment if you know how to set up instruments properly. The better news: the purpose of this article is to show you how to perform synthesis or extraction with the ideal system for your needs.

Synthesis of active pharmaceutical ingredients (APIs)

Synthesis can be performed with several type of instrument set-ups depending on your application. Here is one example of what can be achieved on an instrument you surely already have in your lab: the rotary evaporator. If you need a reflux step for the synthesis, then a reflux condenser can be used on a Rotavapor®. Rotary evaporation is commonly used for sample concentration. But with a Reflux condenser, the rotary evaporator can perform both reflux synthesis and solvent evaporation, giving you best of both worlds. For drug development, you can even have a reflux condenser on an industrial-scale Rotavapor®.

Extraction of pharmaceutical compounds

Extraction is used to separate the desired compounds from the sample matrix, in this case, plant raw material. The factors that influence extraction efficiency include properties of the target analyte, the solvent choice, the applied pressure, temperature and extraction method. Pharma R&D labs are interested in all compounds with beneficial health effects, such as phenolic compounds as flavonoids and phenolic acids with antioxidizing properties, or secondary metabolites with pharmacological effects, such as anti-depressant hypericin extracted from St. John's Wort.

Available extraction methods

Several techniques could be used for the extraction step, such as Soxhlet extraction, maceration, percolation, pressurized solvent extraction (PSE) and more. Multiple system variations could lead you to the desired result. See if the following cases fit your application to find the ideal instrument setup for your needs:

<table>
<tr>
<td>Soxhlet extractions performed seldomly with one sample at a time
↓
Soxhlet glassware

↓
Soxhlet condenser on a Rotavapor® (also possible on industrial-scale level) → ability to perform Soxhlet extraction, reflux and compound concentration offers 3-in-1 usage of your instrument</td>
<td>Extraction of many samples; automation; high recoveries at low extraction temperature
↓
Pressurized solvent extraction – thanks to the applied pressure, cell structures are disrupted leading to high analyte recoveries</td>
</tr>
<tr>
<td>Screening of raw material; many extractions with different parameters in parallel
↓
Highly-automated instrument</td>
<td>Use of different extraction methods; automation capabilities
↓
System capable of handling different extraction methods, such as Soxhlet and Hot extraction</td>
</tr>
</table>

Tip of the application chemist

For an efficient extraction, all you need is the right solvent, a large surface area of your sample and a device you can rely on. Choose a solvent with a similar polarity as the target analyte and make sure it gets in contact with a dry and finely dispersed sample.

Samuel Scheiber
Application Chemist
BUCHI Labortechnik AG

Mr. Samuel Scheiber holds a Master's Degree in Chemical and Bioengineering from the Swiss Federal Institute of Technology (ETH) in Zurich, Switzerland. He joined BUCHI Labortechnik in 2020 as an application chemist for extraction and parallel evaporation.

Publications

Oszmianski et al. 2015. *Determination of Phenolic Compounds and Antioxidant Activity in Leaves from Wild Rubus L. Species*. Molecules 20, 4951-4966.

Al-Ghazali, L. et al. 2021 *The Efficiency of Cyperus rotindus Rhizomes Extract as Antibacterial Agents against some Pathogenic Bacteria*, Sci. J. Med. Research 5 (20) 107-11.

The importance of the middleman

Compound concentration

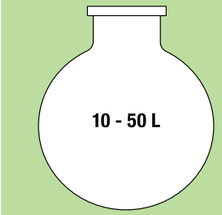
Since synthesis, extraction and purification require a large amount of solvent, a concentration step is required prior to downstream processing. Evaporation is the process typically used to remove solvent and concentrate the compound of interest.

What kind of an evaporation system fits your application?

Case 1

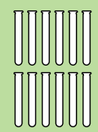
You have done your extraction on a rotary evaporator – use the same instrument, a Rotavapor®, for concentration.

Case 2



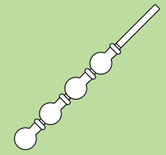
You have large amounts of solvent to evaporate – consider using an industrial Rotavapor® with a flask size ranging from 10 to 50 L. Industrial evaporators can also function with a extraction glassware setup.

Case 3



You need to concentrate multiple samples simultaneously – Use a parallel evaporator to concentrate your extract to a specific volume or to evaporate to dryness increasing your sample throughput.

Case 4



You have a small amount of solvent to concentrate, from 5 to 40 mL – Use a Glass Oven Kugelrohr for concentration of small amounts of valuable samples.

Samuel Scheiber
Application Chemist
BUCHI Labortechnik AG

Mr. Samuel Scheiber holds a Master's Degree in Chemical and Bioengineering from the Swiss Federal Institute of Technology (ETH) in Zurich, Switzerland. He joined BUCHI Labortechnik in 2020 as an application chemist for extraction and parallel evaporation.

Publications

Deng et al. 2020. *Synergistic immunomodulatory effect of complex polysaccharides from seven herbs and their major active fractions.* International Journal of Biological macromolecules, Vol. 165, Part A, 530-541.

Gomes et al. *Feature-Based Molecular Network-Guided Dereplication of Natural Bioactive Products from Leaves of Stryphnodendron pulcherrimum (Willd.).* Metabolites 2021, 11, 281.

How one pharmaceutical biologist combines nature and science in medicine

Assoc. Prof. Dr. Elfahmi earned his PhD in Pharmaceutical Biology from University of Groningen, Netherlands. With nearly 100 publications in journals and conferences, he is currently a Vice Dean of Academic Affairs at The School of Pharmacy, Institut Teknologi Bandung (ITB) in Indonesia, Head of University Centre of Excellence on Nutraceutical and ITB and founder of Markherb. Currently he leads a lab of more than 20 graduate students, post-doctoral fellows and research scientists.

When Dr. Elfahmi successfully defended his doctoral thesis in Netherlands, his supervisor asked him if he was certain he could continue to perform the same research in Indonesia.

"I said I believed I could," recalls Dr. Elfahmi. "Then I came back to Indonesia and we didn't have any instruments for such kind of research topic in my labs. But I went to other labs in the institutes and other universities, first we had to loan, now we have our own. We were able to build up ourselves. We have grown."

Dr. Elfahmi's research into herbal medicine has not just grown, it has exploded. What fuels his fire is his interest in natural products and their benefits for human beings. For as long as he can remember, he has been interested in nature and the history of pharmacy, when most medicine was derived from plant sources. He gives opium, antibiotics, vincristine and podophyllotoxin as examples of common drugs coming from plants and microorganisms and insists nature remains a good source for drug discovery today. Born in Indonesia, one of the most biodiverse locals on Earth, Dr. Elfahmi admits it is no surprise that he has spent half of his life on exploration of natural products.

"I love lab work," adds Dr. Elfahmi. "If I have time, I always go to the lab. I love research, innovation, natural products, discovery of new compounds, active compounds. It's all integrated work. I love to go from extraction, fractionation, purification in the simplest way."

"I love to go from extraction, fractionation, purification in the simplest way."

Read, be optimistic and simplify are his biggest tips for fellow researchers. He credits scientific papers for improving his knowledge, keeping him up to date with latest developments and for giving him fresh ideas. Working efficiently is another must for Dr. Elfahmi. He points out that his lab members have optimized some projects enough to skip chromatography for fractionation, but still achieve high yield quality products. In other cases, they fully rely on their chromatography system, it all depends on the compound at hand. He is also a big fan of biotechnological approaches for production and enhancing and improving stability of metabolites.

"The active compound of a plant is available in very little amounts," says Dr. Elfahmi. "We can apply biotechnology, genetic engineering to improve production. If you want to isolate from plant, you might have to wait for years, if recombinant E. coli or other microorganisms can produce your compounds, you can produce in days. Erythropoietin, a protein therapeutic for example, is a protein produced from human blood. You can take the sequence of the protein, design a synthetic gene, clone into E. coli and E. coli can express the protein and produce it."

Although certainly forward-thinking, Dr. Elfahmi still pays homage to tradition in his work and how he evaluates his success. His favorite publication, "Jamu: Indonesian traditional medicines toward rational phytopharmacological use", took more than 10 years to complete but was worth every minute of hard work.

"Herbal medicine will always remain a great source for drug discovery."

"The review paper about Jamu, an Indonesian traditional medicine system, all Indonesian people know it, I started to write in 2003 and I published it in 2014" explains Dr. Elfahmi. "Jamu started from Jamu Gendong, some plants extracted by boiling water, the fresh jamu is put inside the bottles and stored in bamboo or rattan baskets and a woman would bring it with along wide shawl called "selendang" for carrying the baskets on her back, walk on the street, sell it and consumers drink it for health. The review paper, a lot of people read it, cite it, use it for reference. It has been one of the most cited articles, most read article in "Herbal Medicine" for a few years. And I am happy to bring jamu of Indonesia abroad. People know it, buy it, sell it. I could promote jamu abroad."

And Dr. Elfahmi believes the future of drug discovery lies precisely at this crossing of nature, tradition and medicine. He expects that plant-derived drugs, such as the anti-cancer drug cantharantin, will remain popular, but so will semi-synthetic compounds, where the compound might be initially derived from plant, but is then synthesized at the end of the workflow. In the near future, he believes that herbal medicine can play a key role in the current COVID-19 pandemic, by providing ways for people to stay healthy or even as a potential cure of the disease. And for him, anyone interested in pharmaceutical R&D, can always look at Mother Nature for inspiration.

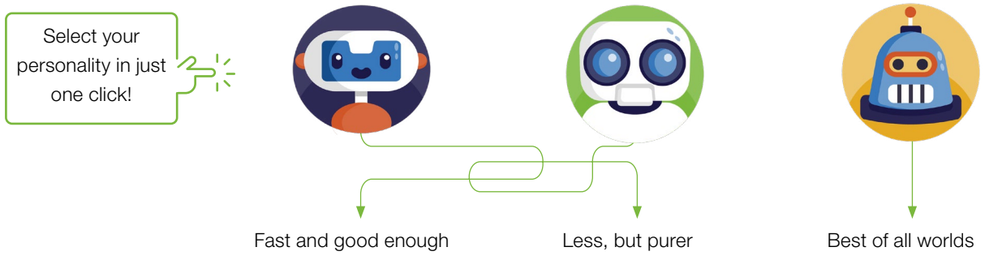
"Herbal medicine will always remain a great source for drug discovery."

3 Ways to obtain pure compounds

What is your ideal preparative chromatography method?

The typical isolation and purification workflow for pharmaceutical compounds involves chemical synthesis or extraction from a natural source. The resulting material is concentrated to reduce sample volume, often followed by chromatography for compound purification and then a secondary concentration step. The final product is analyzed for purity and function by melting point analysis, analytical HPLC, enzymatic assays and other methods.

Scientists in the pharma R&D lab mainly use liquid chromatography to separate a sample, such as a synthesized mixture or a biological crude extract, into single components. The separation process is based on components partitioning between two phases, a stationary phase, typically Silica, with a large surface area and a liquid mobile phase which moves through the stationary phase.



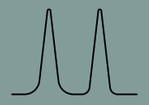
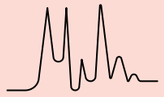
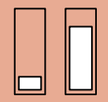
Fast and good enough

If you need to purify large sample quantities at a good resolution, then flash chromatography is the method for you. This medium-pressure technique is ideal as a pre-purification step, as you can load larger sample amounts and still achieve a decent separation.

	Flash
Particle size	15 - 63 µm
Column ID	12 - 115 mm
Flow rate	15 - 250 mL / min
Loading capacity	< 300 g
Max pressure	50 bar

Playing Goldilocks with chromatography systems & consumables

Below is a list of common application-driven examples encountered in the pharmaceutical R&D lab. If you match to one of these, see my recommended conditions for optimal compound separation.

1  **Low-complexity samples** ↓ These show relatively reasonable separation between the peak of interest and impurities. Use medium to large silica particles (15 to 60 µm).	2  **High-complexity samples** ↓ Difficult to separate with partially overlapping peaks. Use small-sized silica particles (5 to 15 µm). These particles offer excellent resolution, but generate high back pressures.	3  **Injection of small sample concentrations (up to 1 g) for high purity results** ↓ Use a prep HPLC system.
4  **Injection of small to high sample concentrations (up to 300g) for decent purity results** ↓ Use a flash chromatography instrument.	5  **Detection of chromophoric compounds** ↓ Use a system with a UV detector. Chromophoric compounds or unsaturated bonds, aromatic groups, or functional groups with heteroatoms absorb light wavelength in the UV or visible wavelength range (200 to 800 nm).	6  **Detection of non-chromophoric compounds** ↓ Use a system with an evaporative light scattering detector (ELSD). Non-chromophoric compounds, such as carbohydrates or lipids, do not absorb light so they cannot be visualized using a UV detector.

Björn Brodbeck
Application Chemist
BUCHI Labortechnik AG

Dr. Björn Brodbeck holds a PhD in inorganic chemistry from Tübingen University, Germany. He has been an application chemist at BUCHI Labortechnik since April 2021, where he specializes in purification.

Publications:

Goebel GL et al. (2022). *European Journal of Medicinal Chemistry. Small molecules with tetrahydroquinoline-containing Povarov scaffolds as inhibitors disrupting the Protein–RNA interaction of LIN28–let-7*

Othman A. et al. (2021). *Natural Product Research. Bassiamide A, a new alkaloid from xero-halophyte Bassia indica Wight*

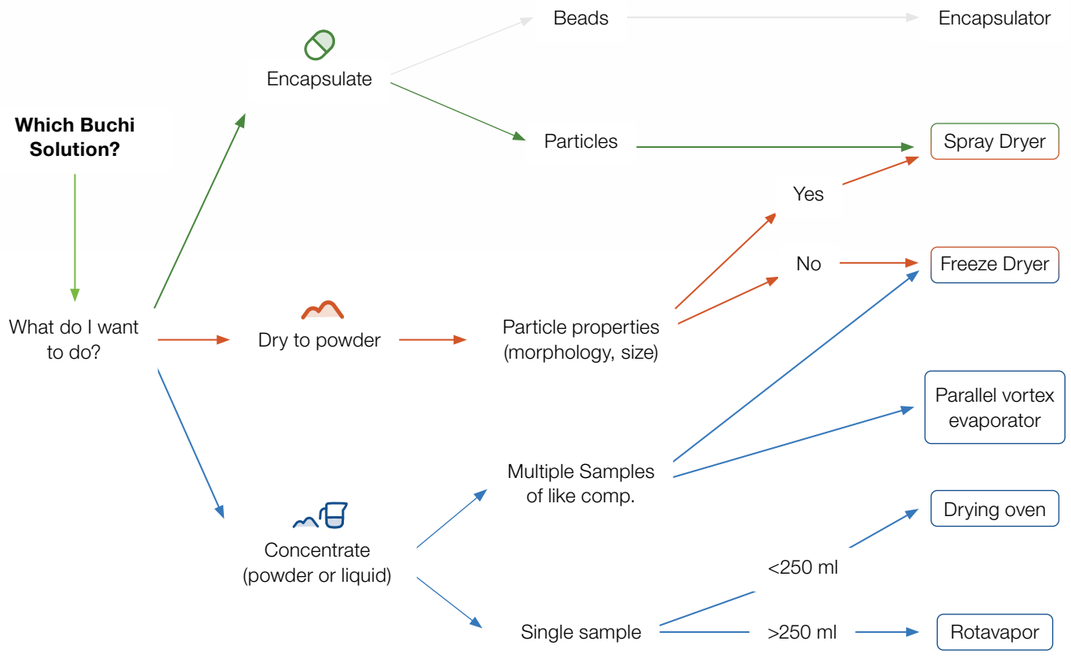
Breaking down the complicated world of solvent removal

To dry or not to dry: not if, but how?

Solvent removal or concentration by drying is an essential process for pharmaceutical applications to facilitate formulation or analysis of compounds of interest. Here, we explore the suitability of freeze dryers, spray dryers, vortex and rotary evaporators for drying purposes in pharma R&D labs.

Drying is the process of separating a liquid from a solid. The vapor is carried away from the solid material and allowed to escape from the drier. In some cases, solvents must be condensed and recovered. In the pharmaceutical R&D lab, evaporation and drying systems are used to remove as much unwanted solvent as possible, while keeping the sample material intact. Rotary evaporation is mainly used in R&D and quality control during synthesis, sample preparation, and extraction processes. Spray drying and freeze drying typically facilitate formulation of final products.

Select your solution in just one click!



Spray drying

This method is widely used for conversion of aqueous or organic solutions, emulsions, dispersions, and suspensions into a dry powder. Spray drying is accomplished by dissolving, emulsifying, or dispersing the core substance in a solvent or in a solution of carrier material. The material is then atomized and sprayed into the drying chamber where a hot stream of drying gas evaporates solvent to produce dry solid particles, which are further separated from the gas stream and collected as a final product.

Spray Drying Tips for Advanced Users:

- If your sample is heat sensitive (ex. probiotics, DNA), use protectant
- If the glass transition temperature is too low, use carrier or protectant to increase it
- If your sample concentration is too low, use a carrier
- If your sample concentration is too high, consider dilution as viscosity may impede sample feeding

- Solvent will determine min. inlet temperature
- Inlet temperature should be set higher to maximize evaporation an d throughput
- Pump should be set as high as possible
- Spray gas should be about 35 mm to have particles that collect well
- Aspirator should be on maximal values to optimize drying

Spray dring, freeze drying and rotary evaporation can all be used with heat sensitive samples to some extand. If that's the case, formulation development should be properly considered and process parameters adapted to protect your sample.

Benjamin Sigmon
Product Manager / Chemist
BUCHI Labortechnik AG

Mr. Benjamin Sigmon earned his B.Sc. in Chemistry from Washington College, USA. He gained further experience in organic chemical syntheses with research positions at Colorado State University and the National Institutes of Health (NIH). He joined BUCHI Corporation as a product specialist in 2020.

Publications

Alhajj N. et al (2021). *Drug Discovery Today. Leucine as an excipient in spray dried powder for inhalation*

Jüptner A and Scherliess R. (2020). *Pharmaceutics. Spray Dried Formulations for Inhalation—Meaningful Characterisation of Powder Properties*

Bakr MM et al. (2020). *Journal of Pharmaceutical Sciences. In Situ Hexosomal Gel as a Promising Tool to Ameliorate the Transnasal Brain Delivery of Vinpocetine: Central Composite Optimization and In Vivo Biodistribution*

User trainings

Unlocking the full potential of lab equipment for drug discovery

Markus Metzler, Head of Global Training at BUCHI Labortechnik AG, highlights the importance of regularly attending courses on pharma R&D techniques and gives insights on how to select the best training for researchers at any level.

Welcome Markus Metzler. To start off, can you tell us a little bit about yourself?

I started working for BUCHI in 1999 when BUCHI acquired Buhler Anatec to extend their NIR instrument portfolio with NIR Technology. After supporting the development of NIR Sales and Service distribution channels, I took over the Customer Support Department which I managed for more than 10 years. Growing demand for training resulted in me taking over a new job title, Head Global Training. In this position, I am responsible for creating and promoting BUCHI's Training programs for affiliates, distribution partners and our customers.

Why are user trainings beneficial for a beginner? For someone with more advanced knowledge?

For beginners, method trainings grant a successful start with their BUCHI solution and ensure the user can take advantage of the full potential of all available system features. If you purchase a Mercedes Benz (or in our case, a BUCHI Rotavapor®), it is not just about driving a car, it is about the experience and the benefits you can take out of the features that are lacking in cheaper models. Importantly for those in pharma R&D, user trainings are often required to be in line with IQ/OQ regulations right from the beginning. Those with advanced knowledge can optimize methods and workflows, save time and money and grow their application knowledge together with chemists and product managers in their field.

How can you assess if a training course is well designed?

Participants can evaluate their course based on degree of interaction, opportunity for experience exchange, networking potential, room for individual questions, proper follow-up, learning success control (didactics) and chance to give feedback. I would also ensure the number of attendees is limited, as learning in a larger group (more than 15) is more difficult. If you are working on challenging samples, the opportunity to bring along your own samples to the training could be very advantageous.

What are some of your most popular user trainings?

We do well in terms of attendance and positive feedback on in-person courses on chromatography, spray drying and NIR Trainings. Our most successful virtual trainings are on spray drying and encapsulation.

Can you recall a positive experience from a user training on R&D methods in pharma labs?

Feedback of participants who attended spray drying & encapsulation trainings come to mind. It is always rewarding to see users beginning to explore the instrument settings, learning how to optimize applications and finally being able to independently create and run methods.

How does a virtual training compare to an in-person training session?

Virtual training can never fully replace in presence training courses (in our business), but online courses will remain in the training landscape after the pandemic ends as their advantages cannot be dismissed (cheap, flexible, no participant limits). However, in my opinion, Blended Learning Approaches, where both virtual and in-person trainings are combined, will increase in popularity in the future.

More courses and trainings.

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Next issue

What is the one factor that can improve the efficiency of your entire drug discovery process? Get your hands on the next issue of “Curiosity” to find out.

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