Liquid-Liquid Chromatography Instrumentation for Laboratory Preparative & Process Chemistry

Liquid-Liquid Chromatography (L-LC) Instrumentation, also referred to as Counter Current Chromatography (CCC, HSCCC, HPCCC), and Centrifugal Partition Chromatography (CPC), designs have existed for 60 years. Solid-Liquid Chromatography (S-LC) techniques include Open Tubular, Flash, MPLC and HPLC also have an extensive history. For the vast majority of applications both L-LC and S-LC have a stationary and a mobile phase. With S-LC the stationary phase is often a liquid, immobilised by bonding onto a solid phase. In L-LC over 99.9% of published applications have one liquid phase stationary, with immobilisation of the stationary liquid phase by the instrument's design operating procedures. S-LC and L-LC therefore have many fundamental similarities. Discussed here are the similarities/differences and significant inter-compatibilities of L-LC and S-LC.

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INTRODUCTION

The use of CCC nomenclature has lead to many decades of confusion in the mode of operation. Non L-LC chromatographers consider a 'Counter Current' mode would involve two liquids moving in different directions. With L-LC/CCC despite the fact they can be used with liquids moving in two opposing directions, in reality for 99.9+% of usage, one phase is stationary and one phase is mobile. The International CCC Committee recently redefined CCC/HSCCC/HPCCC as hydrodynamic CCC and sun/droplet CPC as hydrostatic CCC whilst acknowledging all were Centrifugal Partition Chromatographs (CPC). Maybe this does not resolve the fundamental historic nomenclature confusions? Therefore in this publication, this science is referred to predominately as L-LC, rather than CCC or CPC. Planetary CCC, HSCCC and HPCCC will be described as hydrodynamic L-LC and sun or droplet CPC as hydrostatic L-LC

There are very many fundamentally different modes of L-LC Instrumentation design [1]. Whilst L-LC Instrument designers will discuss amongst themselves that different L-LC concepts may show total incompatibility with other L-LC concepts, regarding selection of resolving biphasic solvents for the same target/matrix, this has been rarely published [2].

The above can even be applied within a single manufacturer's HPCCC or CPC product range, if the manufacturer varies key L-LC design factors. The more key design factors varied within a single manufacturer's HPCCC or CPC product range, the greater the likelihood of failure during Process Scale-up and greater the likelihood of failure in transfer of biphasic solvent choice between different L-LC instrumentation design concepts.

Modular rationalisation of L-LC design such as in the Quattro CCC™ and Partitron CPC™ [2] can reduce the problems of scale-up to make them no more difficult than in S-LC. Modular design allows the massive benefits of L-LC to be explored logically. These benefits include, high sample loading (5 to 15% of utilised L-LC volume, typically a range of 5 to 40g injection, with average closer to 10g per 1000ml capacity for a Quattro L-LC™), reduced solvent usage (typically saving half to tenth solvent requirement to prepare same target mass in same matrix as S-LC), no irreversible 'on-column' adsorption or degradation, no expensive solid phase to poison, each L-LC run can be from infinitely polar to infinitely non polar or visa versa.

The key design factors in hydrodynamic L-LC are sun & planet radii, beta values (even allowing for speed compensation to maintain constant 'G'), rotation speed, coil-winding technique, tubing bore etc. For hydrostatic L-LC the key design parameters are chamber shape/insertions/volume, sun radii and rotation speed.

As previously discussed the more key design factors are changed, the percentage success of process scale-up and cross compatibility between fundamentally different L-LC design modes radically reduces. This factor has to date, not historically been reported in relationship to its percentage occurrence. Disproportionate reporting of successful scale-up/cross compatibility of method transfer of CCC/CPC with different key design modes has led, and continues to lead to distortion of fundamental truths. On occasions it may cause disillusionment of new L-LC users who are unable to reproduce published methods on instrumentation from same or different manufacturers, when the instrument has different key L-LC design modes. Examples of a more realistic situation for scale-up and cross correlation of CCC/CPC, to try to redress the historically bias publications, will be given in the Discussion.

Let us consider S-LC and L-LC from a fundamental chemical/ chromatographic perspective. Given that stationary phase in S-LC is often an immobilised liquid, which has been immobilised by bonding to a solid substrate, it could be considered the only

Figure 1. Components of Quattro CCC

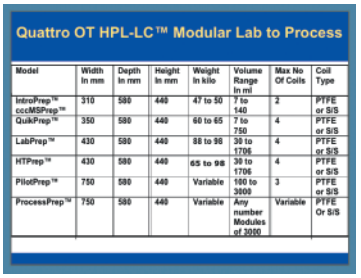

Figure 2. Scale-up Parameters

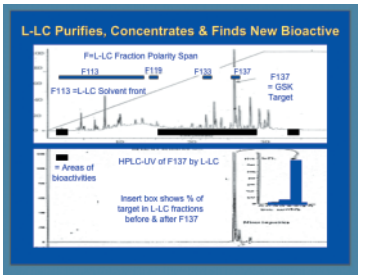

Figure 3. Purification of a new BioActive using L-LC

difference between L-LC and S-LC is that in L-LC, the instrument design maintains one of the pair of immiscible liquids stationary, and in S-LC immobilisation is by adsorption of a liquid onto a solid particle. Both S-LC and L-LC can be used in isocratic and step/linear etc gradient modes [1, 2, 3, 4]. As S-LC and L-LC are potentially similar in chromatography chemistries etc, should they be equally popular and equally understood?

The earlier statement in this Introduction defines the answer; too many fundamentally different designs of L-LC are not mutually compatible. Varying key design parameters in a single concept can lead to excessive scale-up failures.

What then is the solution for successful L-LC design? The one chosen uniquely for the Quattro L-LC™ for hydrodynamic L-LC was to fix as many key L-LC design parameters as possible. The totally modular design, uniquely keeps as many key L-LC design parameters the same as practical, from smallest L-LC MS, 7ml coils to 100+ litre process L-LC capable of multiple tonnes per annum production.

CONTEXT OF MODULAR QUATTRO OPEN TUBULAR, HIGH PERFORMANCE LIQUID-LIQUID CHROMATOGRAPHS™ (OT HPL-LC™).

Figures 1 & 2 show the chassis and coil/volume options of the Quattro L-LC™ model range ('J' Type, Planetary Centrifuge, open, constant id tubing, wound on a planetary bobbin, with no rotating seals). The bobbins (paired planetary rotating bodies, holds the coiled, tubing columns) can have tubing with different material choice. Options include PTFE, Stainless Steel or Titanium. Tubing bore id can vary from 0.5 to 12.5mm, and volumes from 7 to 3000ml for a single rotor assembly. A single bobbin can have two coils. All models except the entry IntroPrep™ have two dynamically balanced bobbins, with up to 4 coils as an option. Each coil can be used independently for same or different preparations, or used in any combination, in series with any coil or multiple of coils of the same id. Uniquely for hydrodynamic L-LC model ranges, all models share the same key L-LC design parameters, inclusive of the same sun and planet radii, speed ranges, beta values, winding techniques and only tubing bore is varied.

This model range is also the only one that allows even the largest bore to be tested on a laboratory based unit, prior to introduction to process based preparation. Hybrid coil winding, that is multiple ids in the same instrument or bobbin, can be custom produced. Multiple bobbin sets for a single chassis are available. In this way the major difficulty with competitor's ranges of needing several different instruments, to validate scale-up, is avoided.

For Process Chromatography, the base module is of 3 litres. Bobbins are interchangeable, and can be exchanged for re-winding if PTFE tubing chosen and cGMP requires virgin material.

Most would use stainless steel or titanium tubing and appropriate cleaning techniques, but renewing PTFE coils is an option. If different bore sizes are required, different paired sets of bobbins may be used. Any number of rotors can be used in series, in parallel or in SMB operations. Clutches and switching valves allow operating mode changes.

RESULTS & DISCUSSION

A discussion of AECS-QuikPrep Ltd Case Studies, completed with a Quattro L-LC™ which demonstrate the considerable benefits, and inter-compatibilities of Open Tubular, High Performance Liquid-Liquid Chromatography™/OT HPL-LC™ and Preparative HPLC in problem solving for a variety of Industries/different applications.

Realism in L-LC for laboratory or process scale-up:

Much of the information that is known on problems of L-LC scale-up and design incompatibility is only discussed verbally within the L-LC community. These negatives are rarely published. We will confirm unpublished Grant funded research ('The Industrial Scale up of Countercurrent Chromatography'. BBSRC/DTI LINK Award Ref: 100/BCE08803. Feb 98 - Jan 00 (£322,668), a collaboration of AECS, Brunel University, University College of Swansea, GSK, Zeneca & Shell Research supported comments by CCC/CPC experts (information acquired verbally) that CCC/CPC of different designs or even a single concept, if we vary the key parameter will on occasions prohibit scale-up. Keeping all parameters the same, only changing tubing bore, certain scale-ups failed. We interpreted the implications of these results so seriously that we embarked on total product redesign of a 7-year prior commercial Quattro CCC™ product. The Quattro L-LC™ post 2000 changed to a unique, modular concept for its whole product range, in order to minimise variability. Since then an additional 9 years has been spent increasing understanding, in order to minimise scale-up failures.

Quattro L-LC™ purifies and concentrates in one step:

During the above LINK Grant project working with GSK the results shown in *Figure 3* were obtained. Two HPLC gradient traces are shown. Top is original HPLC. Below is the HPLC of a single 4ml fraction from a 200+ ml gradient Quattro L-LC™ run. Insert shows the amount of target in fractions before and after the main fraction. Over 90% of target was in one single 4ml fraction. The bars labelled F above top chromatograph show polarity range of L-LC fractions. Apart from solvent front, all show the very small polarity range of OT HPL-LC fractions. An unknown bioactive was found.

Figure 4. Modes of LC as used in NEEM research

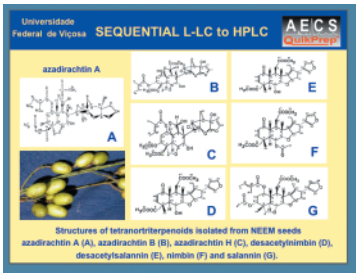

Figure 5. Structures of compounds isolated from NEEM trees

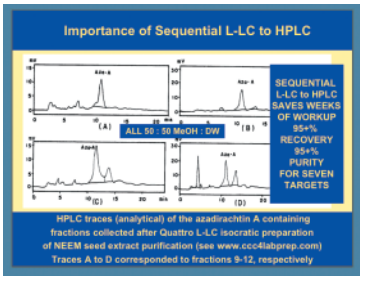

Figure 6. Fractions collected from NEEM seed extract purification

Different design modes of hydrodynamic L-LC (in main only different coil winding techniques) are unable to transfer methodology. Quattro L-LC™ utilisation benefits from Sequential L-LC plus HPLC usage:

The NEEM tree is the Holy Tree of India; it produces such a variety of bioactive targets, that villages in India define it as their Pharmacy. A published method on a competitor's hydrodynamic L-LC could not be transfer to a Quattro L-LC™. The competitor's CCC utilised a different coil-winding mode, but majority of other parameters were comparable. Rapid re-optimisation of biphasic solvent choice for Quattro L-LC™ [5] allowed signification improvement in achieved results over previous methodology. *Figures 4, 5 & 6* show collaborative research with the University of Vicosa, Brasil. Previous to installing the Quattro L-LC™, Professor Gulab Jham took months to prepare just the required amounts of AzA by wet chemistry and S-LC. By Sequential L-LC and HPLC, AzA and six other key related compounds, never prepared in that laboratory before, were prepared in weeks with better than 95% recovery and better than 95% purity [5]. An injection/recovery mass balance was researched, by weighing the dried residue in each L-LC fraction. Within the scope of the method, a full mass balance was obtained. A full mass balance would be an extreme rarity in S-LC for a relatively raw natural product injection; often 20 to 70+ % of the column loading of the total mass injected can be retained if a silica Open Column or Flash Column or HPLC Column is used. Reverse phase columns (ref below) can also irreversibly adsorb or degrade targets. Can it be a surprise that many potentially interesting target compounds are never seen when only S-LC chromatography is utilised, and why L-LC is so often utilised in Natural Product research in preference to S-LC. More exposure of the problems of 'on-column adsorption and degradation' in S-LC could be detailed in the literature to remind researchers of the need for vigilance and open mindedness, in considering the relative merits of S-LC and L-LC.

Different hydrostatic L-LC's (CPC's) design modes having different abilities, and methods used on both are incompatible with a hydrodynamic L-LC, resulting in a method not being transferable from either CPC to Quattro L-LC™. Benefits of Quattro L-LC™ design to significantly enhanced loading relative to CPC's after method re-optimisation:

Deguelin obtained from an Amazonian plant is very valuable (~$20,000/g). Researchers with decades of historic Japanese CPC 1000 ml instrument experience for this separation achieved loading of 150mg per 1000ml CPC capacity. On upgrade to a modern French manufactured 1000 ml CPC using the same method they doubled loadings to 300mg per 1000ml CPC capacity. Their historic method failed on the Quattro CCC. A method developed in less than a day increased loading to 1625mg per 1000ml Quattro L-LC capacity. The client subsequently increased the initial loading to closer to a typical 5 to 40g loading per 1000ml.

Reverse Phase C18 HPLC irreversibly adsorbs/degrades cytotoxic which Quattro L-LC™ prepares:

A Client had a complex bioactive mixture. When it was prepared by reverse phase, end capped C18, preparative HPLC, it had the desired bioactivity. But when the process was transferred to industrial non-HPLC manufacture, the new target mix exhibited extreme cytotoxicity, but by S-LC assay appeared identical. L-LC was used in direct cross correlation to gradient preparative C18, HPLC showed that laboratory studies with end capped, C18 HPLC preparative columns, removed the then unknown cytotoxic compounds, which L-LC methods found. A single multi gram injection onto a 50 x 250mm, 15um C18 column; poisoned the very expensive preparative HPLC column. Multiple L-LC preparations could be run for as many times as required, simply by refilling with liquid stationary phase and reconditioning with mobile phase, plus new injections.

Examples of Wine Research on a Quattro L-LC™ (CCC2006) [6], and HTPrep/Combinatorial research (CCC2008) [7] are available in the literature.

CONCLUSIONS

L-LC compliments HPLC, with narrow range polarity cutting and by helping to find peaks co-eluting in HPLC. L-LC separation are based largely on defining on the polarities of targets, therefore classes of compounds can be separated which can then be optimised without sample loss. These narrow polarity range classes can finally be passed through a HPLC, assuming sample losses can be tolerated. If not, Sequential L-LC to L-LC with different solvents may be utilised. L-LC is a low-pressure technique (typically 100 to 500 psi) thus it can use lower price ancillary equipment than HPLC. L-LC usage has the potential for considerable solvent cost and time savings.

REFERENCES

[1] Conway W.D, Countercurrent Chromatography, Apparatus, Theory & Applications VCH Publishers Inc, 1990

[2] Leitao G.G, El-Adji S.S, Melo, W.A., Leitao, S.G, Brown, L, J. Liq. Chromatogr. Relat. Technol. (2005) 28, 2041-2051

[3] Leitao G.G, Souza, P.A, Moraes, A.A, Brown, L, J. Liq. Chromatogr. Relat. Technol. (2005) 28, 2053-2060

[4] Comprehensive Analytical Chemistry XXXVIII, A Berthod (Ed) (2002), Countercurrent Chromatography, Chapter 2, Berthod A, Brown L, Leitao G.G, Leitao. S.G.

[5] Silva J.C.T, Jham G.N., Oliveira, R.D. L., Brown, L, J of Chrom A (2007), 1151, 203-210

[6] Vidal S, Hayasaka, Y, Meudec, E., Cheyner, V, Skouroumounis, G, J.Agric. Food Chem. (2004), 52, 713-719

[7] Wangenaar F.I, Hochlowski, J.E, Pan, J.Y, Tu, N.P, Searle, P.A, J Chrom A, 1216 (2009) 4154 – 4160.