In-silico prediction of retention times as a strategy to avoid matrix effects in LC-MS/MS bioanalysis

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It is established that for electrospray LC-MS(/MS) assays, co-eluting phospholipids may cause ion suppression which can lead to method inconsistency and reduced sensitivity [1, 2]. The abundance of phospholipids in cell membranes means they are a common contaminant of analyses involving plasma extraction. Therefore, avoiding co-elution of phospholipids and analytes during a chromatographic run, or removing phospholipids completely during sample preparation is highly desirable. Typically the method development process for an LC-MS/MS bioanalytical assay follows the sequence of MS-ion optimisation, development of LC conditions, MS source optimization and then development of extraction. It is only at this stage that the effectiveness of sample clean-up combined with chromatographic selectivity is demonstrated. If a substantial matrix effect or interference is present, it may be necessary to re-develop the LC method completely, which is both resource and cost inefficient.

Various computer software packages are commercially available that predict chromatographic retention times (t_R). One such application is ACDLabs/LC Simulator from Advanced Chemistry Development, Inc. (ACDLabs, Toronto, Canada). LC simulator uses experimentally determined t_R values to build a predictive model of a given chromatographic system, based on structural analysis and Log D prediction. The model can then be used to predict the retention time, under the same particular experimental conditions, for any new compounds with which the user challenges the software with. Theoretically, predicting analyte t_R and phospholipid t_R, prior to any experimental lab work, should streamline the method development process by highlighting potential co-elution and hence matrix effects.

Work was undertaken in our laboratories to build a chromatographic model based on the retention times of a representative set of pharmaceutical compounds and phospholipid reference standards. The resulting model allowed us to predict the retention time of any new drug in relation to the most abundant phospholipids – glycerophosphocholines (GPCho) from plasma extracts.

LC-MS/MS conditions

An ACDLabs/LC Simulator model was built using twenty small molecule analytes, representing a range of drugs typically seen in bioanalysis, and eight lyso-GPCho and GPCho reference standards. The LC system used throughout this work was a Waters ACQUITY UPLC® running a commonly used 2.5 minute scouting gradient of acetonitrile / water / 0.1% (v/v) formic acid. Models of several stationary phases have been created but a Waters 50 x 2.1mm 1.7µm BEH Phenyl proved to be the most versatile for this particular combination of analytes. Both BEH C_{18} and HSS T3 (C_{18}) phases retained the phospholipids to such an extent that their chromatographic peaks were broad and poorly resolved. This illustrates the problem of phospholipid build-up on reverse-phase columns, where phospholipids that are not completely eluted can build up on a column and elute unexpectedly in any subsequent analytical runs [3]. LC-MS/MS detection was by both positive and negative TurboIonspray™ MRM on an API 4000, API 5000 or AB Sciex 5500 QTrap.

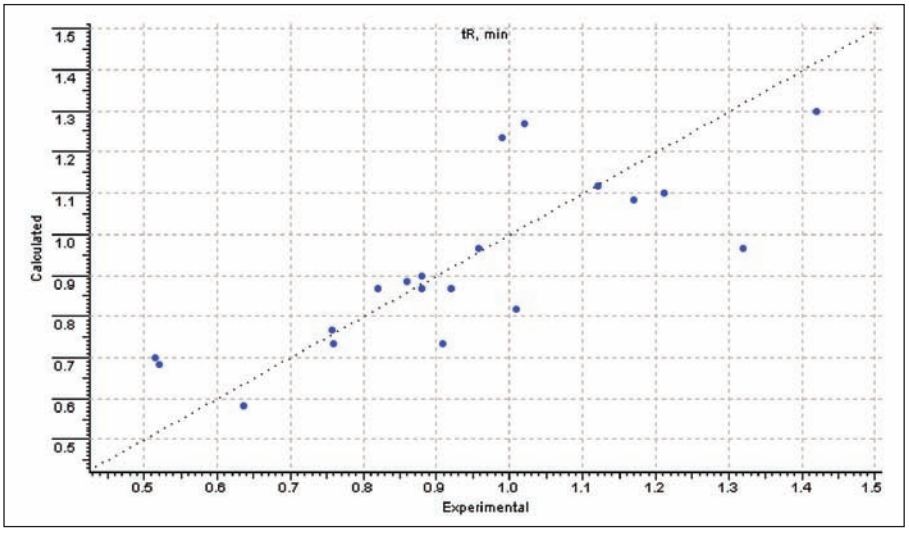

Figure 1. A plot of experimental retention time versus retention time calculated by ACDLabs/LC Simulator for 20 small molecules, R = 0.80. Experimental tR values were from the Waters Acquity phenyl column with MeCN/water/0.1% formic acid.

Materials

The small molecule compounds were as follows: Scopolamine, Nikethamide, Ketamine, Phenazone, Pethidine, Isoxsuprine, Butorphanol, Propranolol, Nordazepam, Acepromazine, Amitriptyline, Naloxone, Meptazinol, Pimobendan, Clopidogrel acid, Omeprazole, Hydrocortisone, Cortol, Clopidogrel and Nitisinone.

The phospholipid standards were 1-hexadecanoyl-sn-glycero-3-phosphocholine (lyso-GPCho 16:0), 1-octadecanoyl-sn-glycero-3-phosphocholine (lyso-GPCho 18:0), 1-cis-9-octadecenoyl-sn-glycero-3-phosphocholine (lyso-GPCho 18:1), 1,2-ditetradecanoyl-rac-glycerol-3-phosphocholine (GPCho 14:0, 14:0), 2,3-dihexadecanoyl-sn-glycero-1-phosphocholine (GPCho 16:0, 16:0), 1-cis-9-octadecenoyl-2-hexadecanoyl-sn-glycero-3-phosphocholine (GPCho 18:1, 16:0), 1,2-dioctadecanoyl-rac-glycero-3-phosphocholine (GPCho 18:0, 18:0) and 1,2-di-(cis-9-octadecenoyl)-sn-glycero-3-phosphocholine (GPCho 18:1, 18:1).

Results

Initially two models were generated – one for the small molecules and one for the phospholipids. The accuracy of small molecule prediction was assessed by generating a model using eleven of the twenty small molecule compounds and then predicting the t_R of the remaining nine. Comparing this predicted t_R with experimental t_R showed a moderate level of accuracy, with differences ranging from -0.30 minutes to +0.41 minutes (median of +0.097 minutes, standard deviation 0.24). The software provides a measure of accuracy based on a plot of experimental t_R vs calculated t_R, which gives a correlation coefficient (R) of 0.8674, standard deviation 0.084. Using all 20 experimental t_R values gives a model with an R of 0.7999, standard deviation 0.097 as shown in Figure 1.

The phospholipid model was built using the experimental t_R values for eight lyso-GPCho and GPCho reference standards. In this case the model was more accurate with an R value of 0.9944, standard deviation 0.009. This improvement is not surprising since the model is built using structurally similar compounds, which is easier for the software to manage.

The accuracy of the phospholipid model was assessed by predicting the retention times of all possible lyso-GPCho and GPCho compounds identified in human plasma by the study of Pang *et al* [4]. The predicted chromatogram was compared to a plasma sample extracted by protein precipitation with acetonitrile (ratio 1:3). The phospholipids in the extract were detected by monitoring the SRM transition m/z 184→184, which detects the trimethylammonium-ethyl phosphate cation, specific for phospholipids with the choline head group [1, 5, 6]. The similarity between the predicted chromatogram (Figure 2) and the measured extract (Figure 3) is striking and suggested the model behaved well.

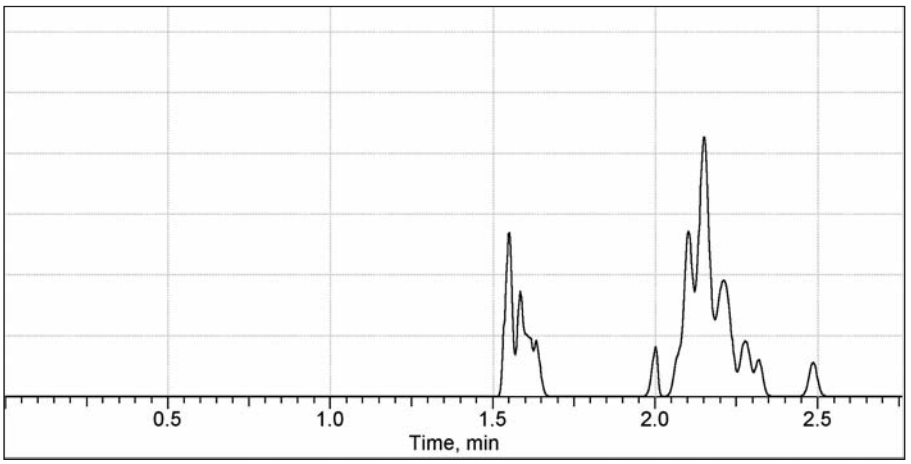

Figure 2. Chromatogram of 30 lyso-GPCho and GPCho phospholipids as predicted by ACDLabs/LC Simulator under gradient elution on the Waters Acquity phenyl column with MeCN/water/0.1% formic acid.

Conclusion

The final tool developed is a prediction model, built using the representative test compounds and phospholipid standards, which is used to guide method development, the most technically demanding and time-consuming stage of bioanalysis. By putting any new analyte through the combined chromatographic prediction models there is potential to speed up the method development process by determining a suitable (UP)LC starting point and/or by acting as an early warning system if a compound co-elutes with phospholipids, meaning that extensive clean up is required if it can't be chromatographically separated. ACD/LC Simulator software predicts t_R of drugs to a degree of accuracy very usable for the avoidance of matrix effects.

An important factor to consider is that not all matrix effects are due to phospholipids. Salts can cause significant suppression but are mainly avoided by developing a method with a sensible retention factor (k'), since they tend to elute very close to the solvent front. Also, it is not uncommon to find that test samples from preclinical studies contain a significant amount of a dose vehicle, such as TWEEN 80 or PEG400, which are known to cause significant disruption of in-source ionisation [7, 8]. Studies have also shown that samples stored in particular tubes are also known to cause problems due to co-elution with leached material [9]. We are currently investigating these other potential sources of interference and will add this information into the final model.

One possible drawback for the final prediction model is that no account is made as to how well a particular compound ionizes in a mass-spectrometer under the given LC conditions. For example, changing the mobile phase pH in the model may separate an analyte from the phospholipids, but might be detrimental to overall sensitivity. In these cases the typical method development strategy of practical testing and pragmatism will come into play.

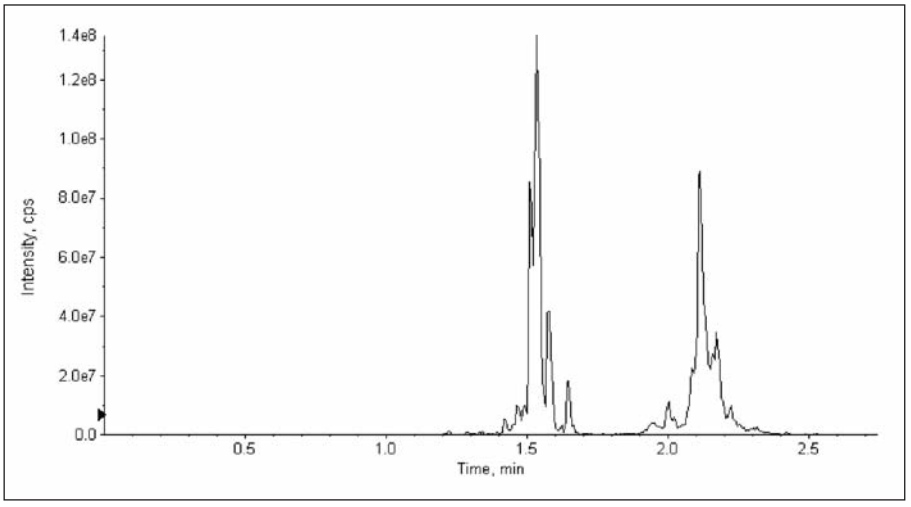

Figure 3. Chromatogram of human plasma extracted by protein precipitation, monitoring for phospholipids with the choline head group.

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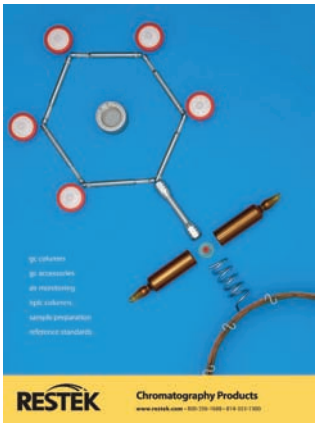

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