アプリケーションノート

Peptide Mapping with Enhanced Chromatographic Resolution and Mass Accuracy

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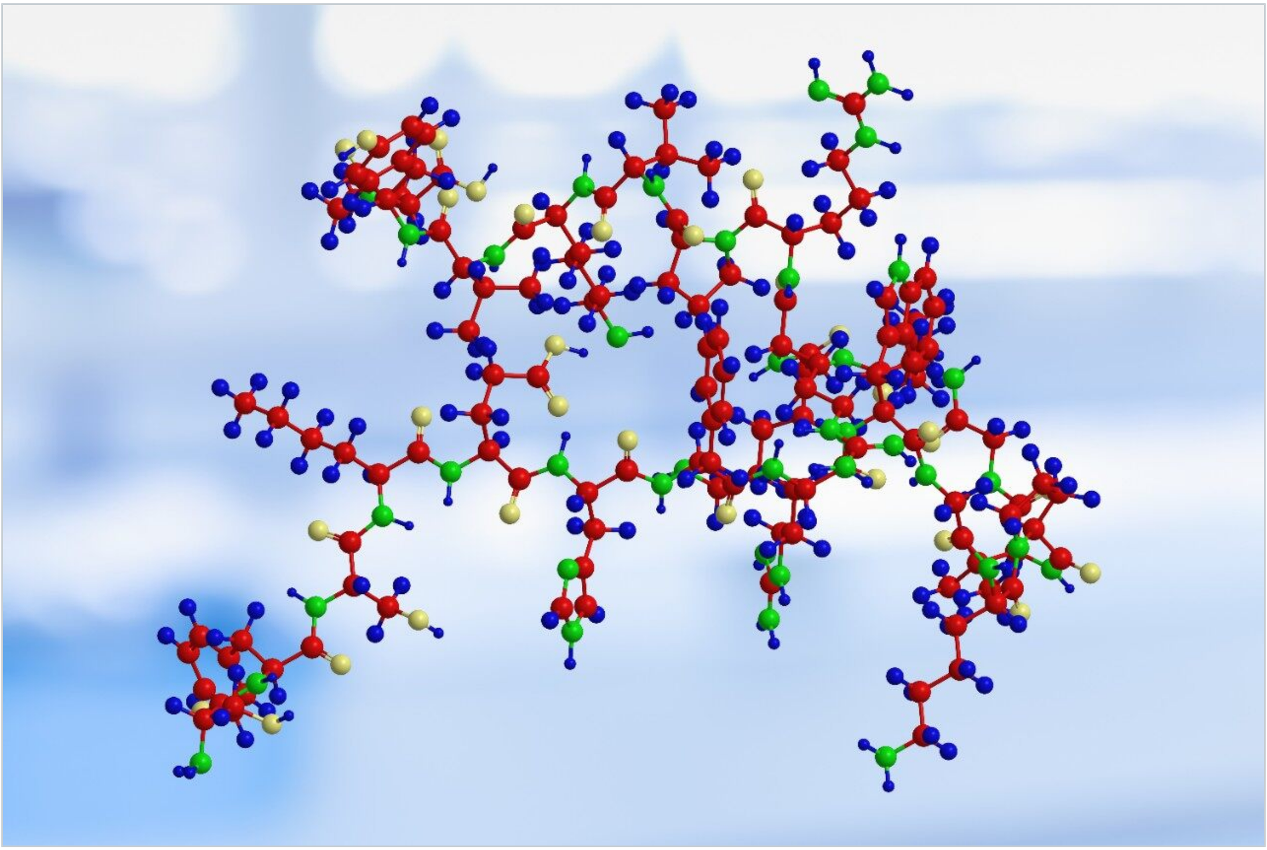


Abstract

In this application note, we present a case study for the optimization of tryptic peptide mapping for a model protein such as phosphorylase B from *Oryctolagus cuniculus* (rabbit). Results on the identification of Lyc-C fragments of delta hemoglobin in the presence of other hemoglobin chains from human blood are shown as well. All data was acquired using a Waters LCT Premier oa-TOF MS in conjunction with ACQUITY UPLC.

Benefits

- With the advent of UPLC, separations of peptide mixtures can be carried out faster while still maintaining excellent separation power and thus giving a high level of peptide coverage
- The combination of both UPLC and TOF provides an unsurpassed level of analytical power that greatly enhances peptide mapping studies

Introduction

Peptide mapping by means of LC/UV and/or LC-MS is an invaluable tool in analysis and quality control of biotherapeutic proteins. Peptide mapping has been widely used for purity testing, comparability testing and batch release of protein therapeutics. Through the implementation and now routine use of electrospray ionization and mass spectrometry (MS) over recent years, the field of protein chemistry has been revolutionized.

High resolution MS provided by bench-top orthogonal acceleration time-of-flight (oa-TOF) MS instrumentation has become widely used for protein chemistry analysis. MS yields important information about peptide sequence and can be used to detect and confirm post-translational modifications. The use of MS provides an additional dimension to that afforded by just chromatographic separation alone, and hence mass spectrometry is used in conjunction with conventional LC/UV peptide mapping to provide an extra level of analytical specificity. However, through the use of time-of-flight instrumentation, data quality can be further enhanced to give high accuracy information provided by exact mass measurement. The coupling of oa-TOF MS technology with the new advances in UltraPerformance LC (UPLC) now provides peptide chemists with a powerful and unique analytical tool giving unsurpassed analytical results.

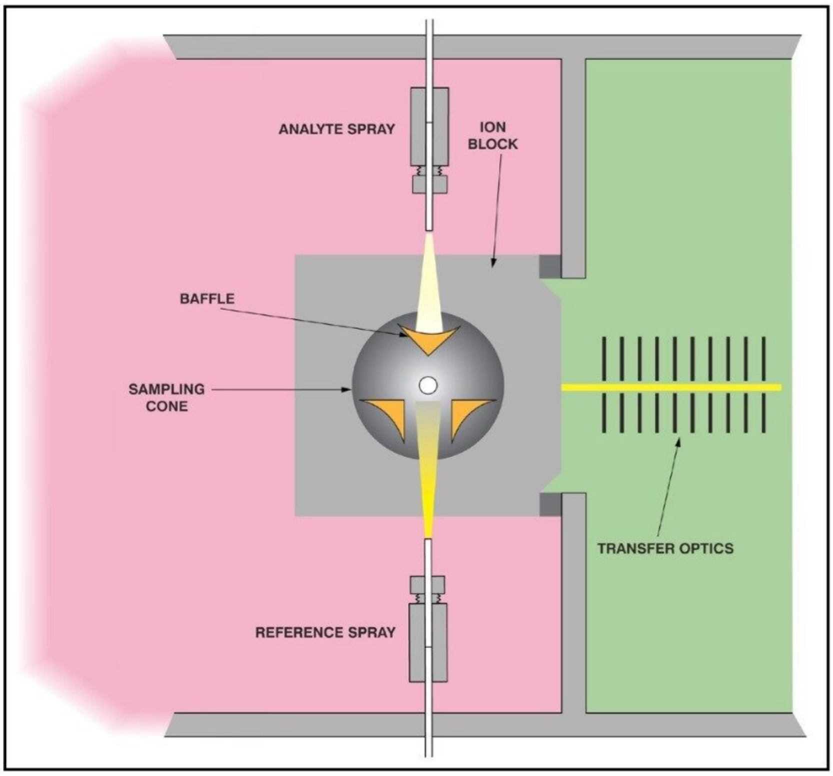
In this application note, we present a case study for the optimization of tryptic peptide mapping for a model protein such as phosphorylase B from *Oryctolagus cuniculus* (rabbit). Results on the identification of Lys-C fragments of delta hemoglobin in the presence of other hemoglobin chains from human blood are shown as well. All data was acquired using a Waters LCT Premier oa-TOF MS in conjunction with ACQUITY UPLC.

The Waters ACQUITY UPLC System and LCT Premier Mass Spectrometer.

Experimental

The digest samples were analyzed by LC-MS. The samples – when required – were diluted with an aqueous 0.1% formic acid solution to an appropriate concentration. A Waters ACQUITY UPLC binary solvent manager was used to perform the UPLC separation with the column outlet directly connected to the source of the mass spectrometer. The analytical column was a 10 cm x 1 mm ACQUITY BEH C_{18} 1.7 µm Column that was operated at a flow rate of 150 µL/min. Mobile phase A comprised of 0.1% formic acid in water and mobile phase B of 0.1% formic acid in acetonitrile. The gradient was from 5 to 30% in 15 min.

MS data was acquired using an LCT Premier oa-TOF mass spectrometer operating with positive electrospray ionization. The TOF analyzer was calibrated with a sodium formate solution. A 100 fmol/µL [Glu1]- Fibrinopeptide B solution was used as a ‘lockmass’ for exact mass measurement and was introduced into the mass spectrometer via the reference sprayer of the LockSpray ion source at a flow rate of 2 µL/min. LockSpray provides the ability to introduce a reference mass over long periods of time without interference from the analyte data. Having this capability provides a simple experimental setup for exact mass measurement whilst maintaining high quality data.



Schematic Diagram of the LockSpray Ion Source.

Results and Discussion

Peptide mapping mass accuracy feasibility study

A 5 picomole amount of a MassPREP tryptic digest solution of phosphorylase B was injected on-column and used to optimize chromatographic resolution. The tryptic LC-MS map is shown in Figure 1, and shows a high degree of peak separation even with a relatively short run time. From the chromatogram, 35 unmodified peptides were selected and mass measured to determine the RMS mass measurement error. The latter was conducted automatically with the all file accurate mass measure option of MassLynx 4.1 Software. Missed cleavages were not considered for the mass accuracy calculations and the results are summarized in Table 1.

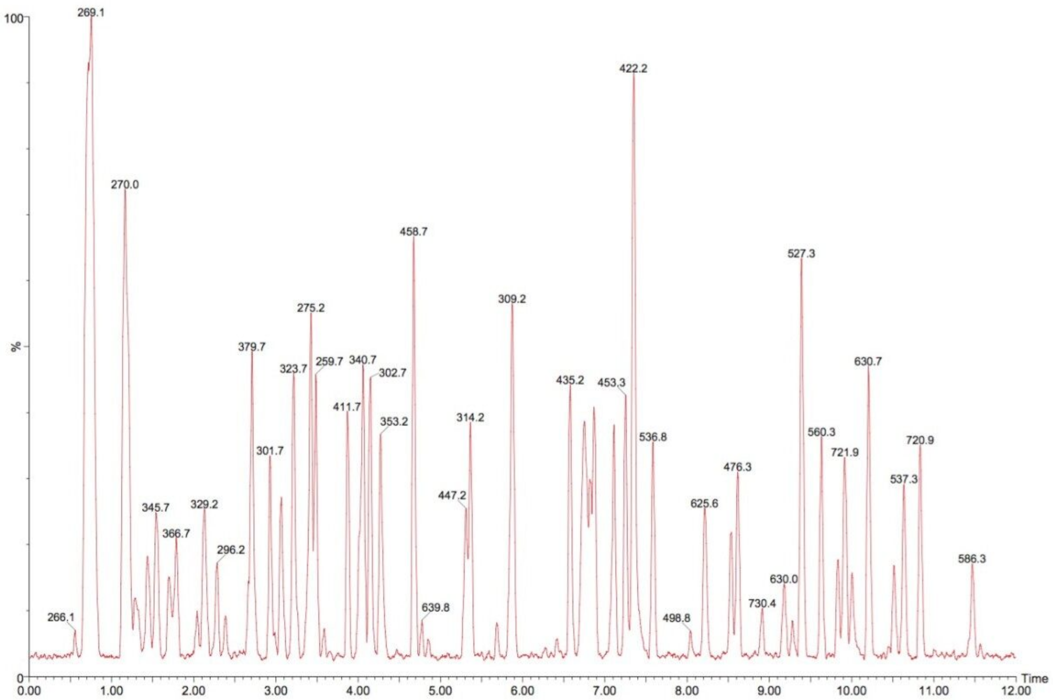


Figure 1. LC-MS separation of tryptic digested Oryctolagus cuniculus (rabbit) phosphorylase B.

mass (M+ H)+	m/z (1+) observed	m/z (2+) observed	m/z (3+) observed	ppm	tr (min)	peptide sequence
4896.2495						TLQNTMVNLALENACDEATYQLGLDMEELEEIHEDAGLGNGGLGR
3712.6790						FMLNGALTIGTMDGANVEMAEEAGEENFFIFGMR
2448.1922			816.7351		9.84	VIPAADLSEQISTAGTEASGTGNMK**
2307.1478			769.7192		13.38	WVDTQVVLAMPYDIPVPGYR**
2334.1620						LAACFLDSMATLGLAAYGYGIR
2118.1375			706.7188		12.54	VAIQLNDTHPSLAIPELMR**
1991.0061		996.0082	664.3389		8.04	INMAHLCIAGSHAVNGVAR**
1890.0079		945.5066		-1.32	10.20	LITAIGDVVNHDPVVGDR*
1874.9065			625.6399		8.22	TCAYTNHTVLPEALER**
1865.7945			622.5878		11.85	ICGGWQMEEADDWLR**
1853.9942			618.6622		11.43	WLVLCNPGLAEIAER**
1765.9483						QEYFVVAATLQDIIR
1756.9527			586.3230		11.47	QLLNCLHVITLYNR**
1689.8859			563.9660		10.52	ARPEFTLPVHFYGR**
1609.8696			537.2953		10.64	VHINPNSLFDVQVK**
1580.8318						QIEQLSSGFFSPK
1566.7910		783.8979	522.9311		14.80	DFNVGGYIQAVLDR**
1554.7661						IYLSLEFYMGR
1550.7696		775.8936			10.63	IGEEYISDLDQLR**
1473.8576			491.9561		14.04	WPVHLLETLLPR**
1442.6950		721.8532		2.49	9.91	VLYPNDNFFEGK*
1440.7369		720.8748		3.40	10.83	LLSYVDDEAFIR*
1426.7801		713.8975		4.97	8.61	HLQIIYEINQR*
1380.6511			460.8906		9.71	DIVNMLMHHDR**
1355.6742			452.5621		9.41	DYYFALAHTVR**
1278.5385		639.7759			4.78	GYNAQEYYDR**
1262.5939		631.8039		4.83	8.53	VFADYEEYVK*
1229.6735		615.3425			8.52	GLAGVENVTELK**
1177.5524		589.2854	393.1902		7.22	DFYELEPHK**
1145.5626		573.2856		0.70	10.02	YEFGIFNQK*
1117.5636		559.2878		3.75	6.74	VAAAFPGDVDR*
1096.5058						TQQHYYEK**
1072.5421		536.7740		-1.77	7.58	EIWGVEPSR*
1062.5499		531.7824		6.68	6.81	MSLVEEGAVK*
1059.5429						SRPLSDQEK
1054.4840		527.7468		1.71	7.10	TNFDAFPDK*
1053.5727		527.2909		1.23	9.39	VIFLENYR*
994.5832			332.1995		6.85	HLHFTLVK**
956.4796			319.5027		1.56	VEHTSQGAK**
947.4727		474.2397		-1.16	4.01	NNVVNTMR*
945.4611		473.2351		1.37	2.98	AAPGYHMAK*
918.4679		459.7395		3.59	5.87	APNDFNLK*
916.4846		458.7482		4.36	4.67	NLAENISR*
893.4152		447.2118		0.67	5.31	YGNPWEK*
869.4515		435.2299		0.57	6.57	FAAYLER*
843.4934		422.2503		-0.71	7.35	VLVDLER*
839.4985		420.2565			4.31	IHSHLK**
832.4563		416.7349		6.84	6.86	AWEVTVK*
822.4468		411.7260		-3.16	3.87	TIAQYAR*
769.4090		385.2113			3.49	LPAPDEK**
758.4155		379.7135		4.87	2.71	TNGITPR*
747.4035		374.2086			6.26	QPDLFK**
732.3522		366.6805		1.36	1.79	VEDVDR*
705.3963		353.2021		0.14	4.26	TVMIGGK*
696.3787		348.6938		1.58	2.14	DHLVGR*
690.3781		345.6931		0.43	1.54	NIATSGK*
689.3617		345.1861			1.39	IHEYK
680.3977		340.7023		-1.32	4.05	VSALYK*
676.3300		676.3419			11.74	LDWDK
657.3678		329.1875		-0.91	2.14	NVATPR*
646.3770		323.6922		-0.62	3.21	VSLAEK*
627.3824		314.1949		-0.64	5.36	IPELR*
617.3769		309.1928			5.88	FVVPR**
611.2783		306.1435			1.29	FSSDR**
604.3453		302.6768		0.83	4.15	LWSAK*
602.3620		301.6838		-3.65	2.92	QISVR*
592.2508		296.2569			2.29	CQER**
591.2885		296.1481		-0.17	2.27	EWIR*
550.2732		275.6399		-2.18	1.43	NFNR*
549.3143		275.1620		3.45	3.42	FLNR*
539.2395	539.2477				1.75	FGCR**
536.2827	536.2978	268.7290			0.55	FQNK
531.2885	531.2978	266.2250			0.55	LDQR
518.3119		259.6609		4.04	3.48	MVIR*
518.2569	518.3250	259.6560			3.48	QENK**
508.3129	508.3184	254.7680			4.02	TIFK**
504.3214	504.3319	252.7695			4.16	MIIK**
487.2500						EPNK**
486.2598	487.2720				1.08	DPVR**

1 µL of digested human serum sample was injected on column, which corresponded to approximately a 30pmol load of total protein digest. The main hemoglobin components in human serum comprise of the alpha and beta chain and to a lesser extent the delta and gamma chains. The LC-MS chromatograms of the Lys-C digest of this sample is shown in Figure 3(a). The peptides of interest originate from the delta chain and are present at low concentrations resulting in reduced electrospray signal intensities. Due to the low ion currents produced, statistics challenged the exact mass measurement analysis. Nevertheless, the RMS error for the three peptides of interest was found to be better than 4.6 ppm, even at very low ion currents. Shown in Figure 3(b) is one of the obtained peptide spectra (L4).

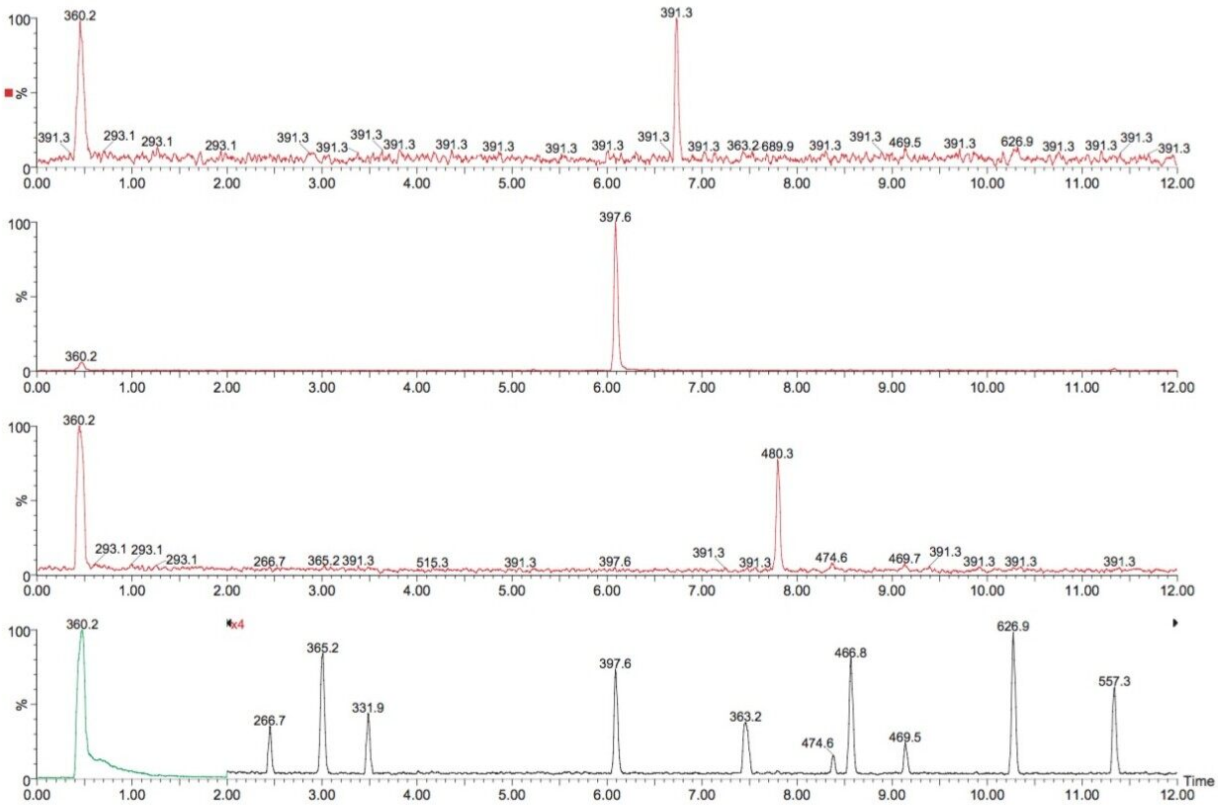


Figure 3 (a). LC-MS separation of Lyc-C digested human hemoglobin chains. The lower trace shows the base peak intensity chromatogram. The three top traces are the mass extracted chromatograms of the hemoglobin delta chain of interest for m/z 391.3, m/z 397.6 and m/z 480.3, respectively.

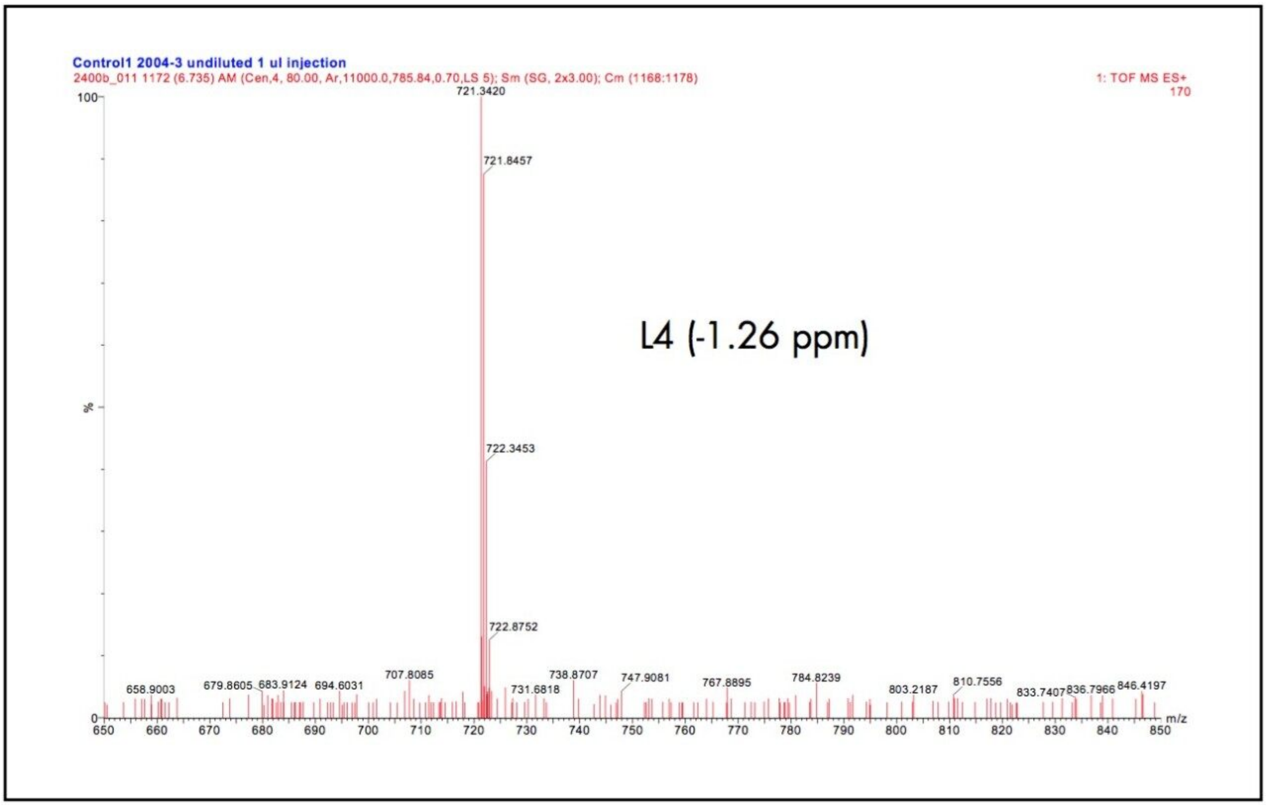


Figure 3 (b). Spectrum of the L4 fragment (-1.26 ppm) of human hemoglobin delta chain.

Conclusion

The aim of this application note was to show that the use of UPLC coupled directly to time-of-flight mass spectrometry has a significant benefit for the analysis of peptides. The facility of exact mass measurement provided by time-of-flight MS greatly enhances the analytical answer produced and provides an extra level of confidence when determining analytes of interests. In addition to sensitive full spectral acquisitions, exact mass measured data is produced routinely providing a high degree of analytical specificity. Hence, the method benefits from the orthogonal separation on *m/z* of co-eluting species by means of oa-Tof mass analysis. Through the use of LockSpray, the practical aspects associated with exact mass measurement are made easy with excellent reliability. With the advent of UPLC, separations of peptide mixtures can be carried out faster whilst still maintaining excellent separation power and thus giving a high level of peptide coverage. The combination of both UPLC and TOF provides an unsurpassed level of analytical power that greatly enhances peptide mapping studies.

Acknowledgements

ECMP, Laboratoire de Spectrometrie de masse bioorganique, Strasbourg, France is kindly acknowledged for providing the Lys-C digest of human hemoglobins.

References

1. J.R. Mazzeo, T.E. Wheat, B.E. Gillece-Castro and Z. Li, Enabling Significant Improvements for Peptide Mapping with UPLC 720001339EN <https://www.waters.com/webassets/cms/library/docs/720001339en.pdf> .

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720001830, August 2006