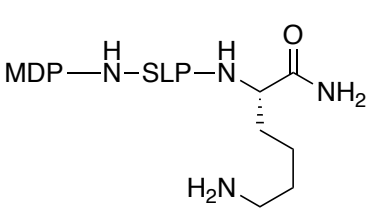
Dual Synthetic Peptide Conjugate Vaccine Simultaneously Triggers TLR2 and NOD2 and Activates Human Dendritic Cells

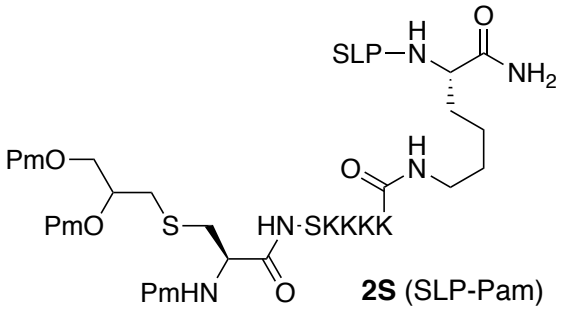
Gijs G. Zom[†], Marian M. J. H. P Willems[‡], Nico J. Meeuwenoord[‡], Niels R. M. Reintjens[‡], Elena Tondini, Selina Khan[†], Herman S. Overkleeft[‡], Gijsbert A. van der Marel[‡], Jeroen D. C. Codee[‡], Ferry Ossendorp*[†], and Dmitri V.Filippov*[‡]

[†]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, P.O. Box 9600, 2300 RC, Leiden, The Netherlands

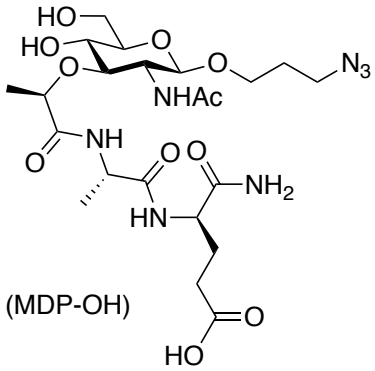
[‡] Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands



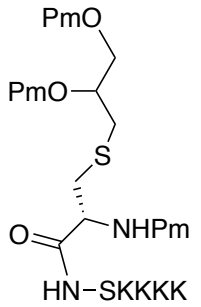
1S (MDP-SLP)



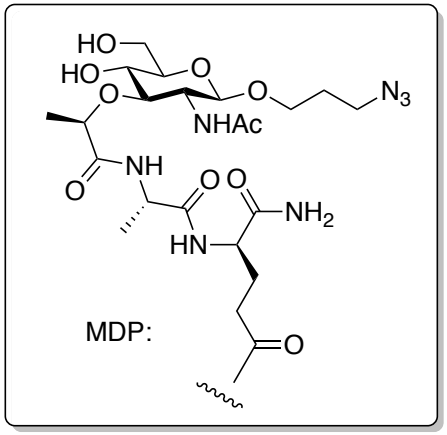
2S (SLP-Pam)

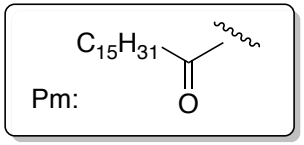


3S (MDP-OH)



4S PamCSK$_4$

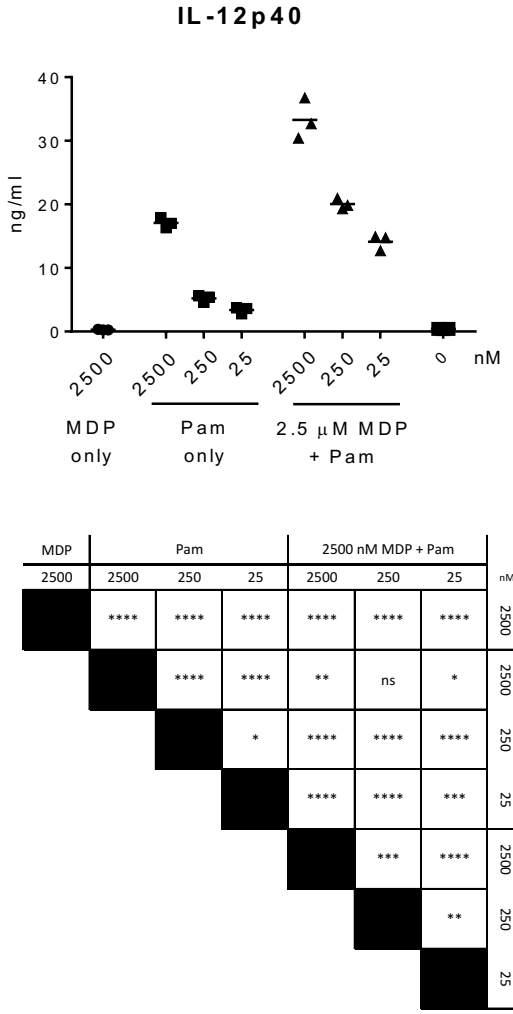




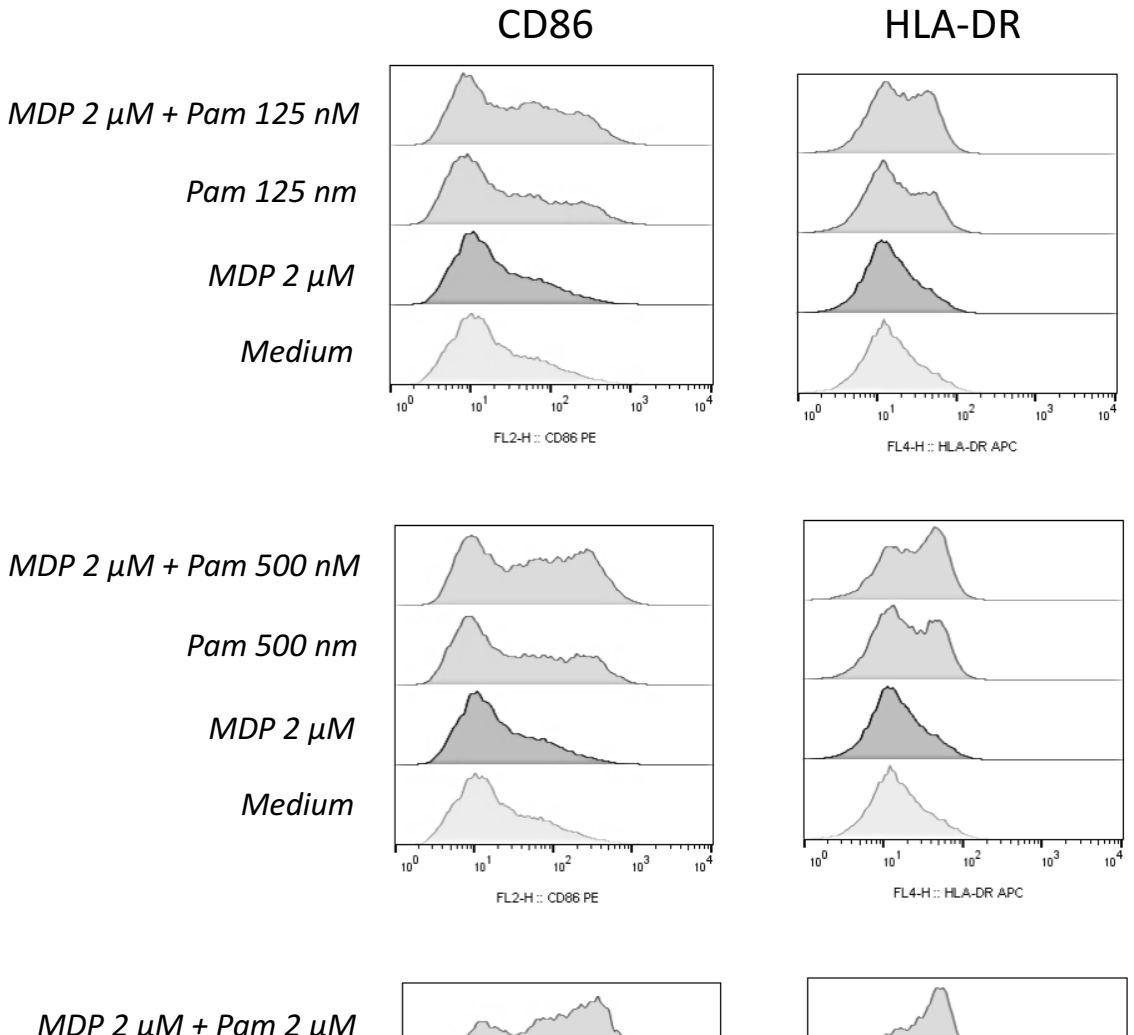
SLP = DEVSGLEQLESIINFEKLAAAAA

Supplementary Figure S1. Essential control compounds used in the immunological evaluation of the dual conjugates.

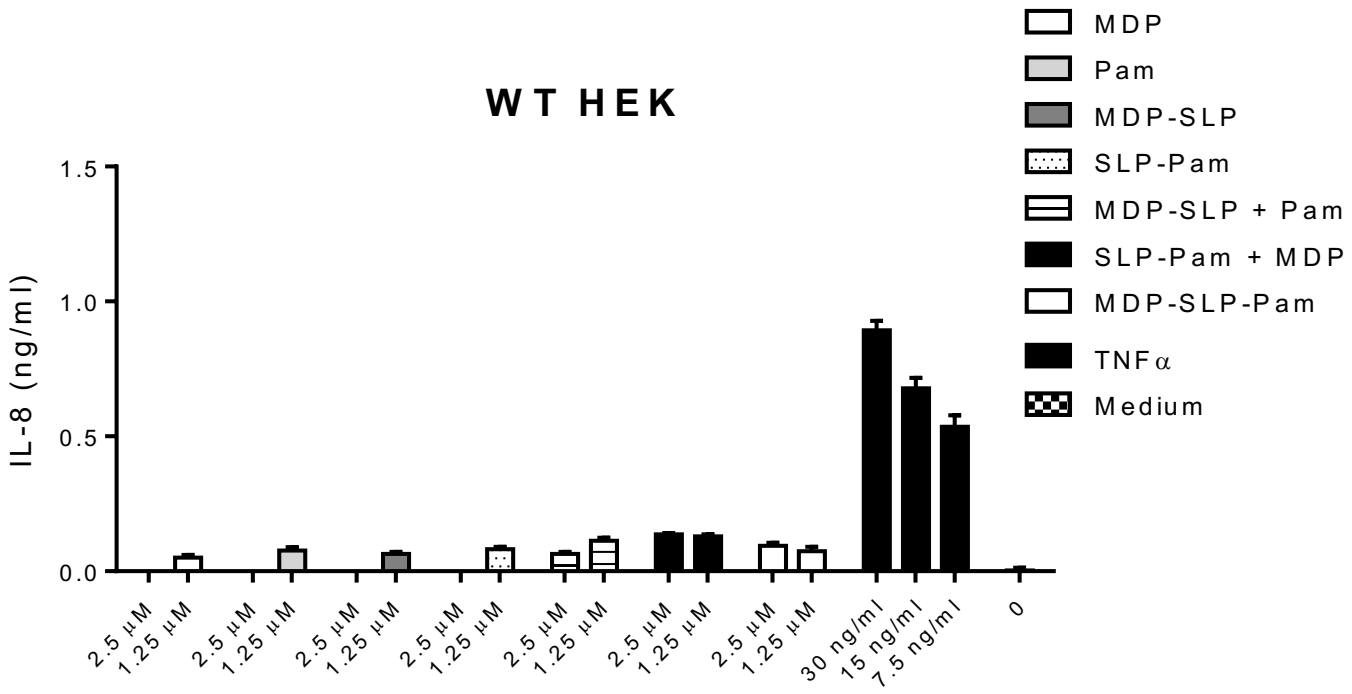
A



B

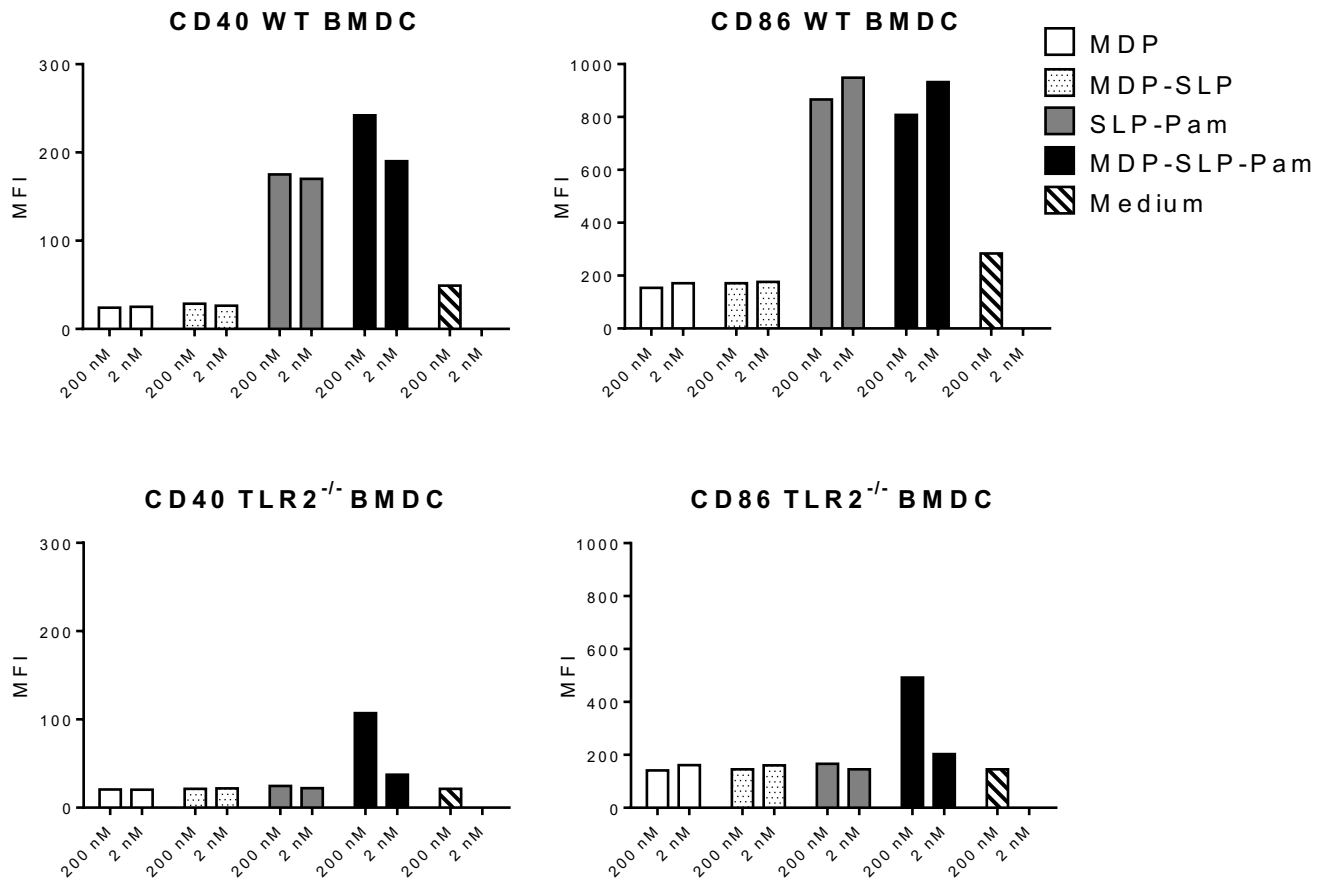


Supplementary Figure S2. (A) Concentration of IL-12p40 in the supernatant of human moDCs stimulated overnight with either 2.5 µM MDP, or different concentrations of Pam3CSK4 (Pam), or 2.5 µM MDP combined with Pam3CSK4 in different concentrations. Summary of calculated significance below the figure. Significance determined by one-way ANOVA and Bonferroni post-hoc test. ns: not significantly different; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (B) Flow cytometry histograms showing the expression of CD86 and HLA-DR on monocyte-derived DCs after overnight stimulation with the indicated constructs. Three stimulation dosages are shown: top 125 nM, middle 500 nM, bottom 2 µM. Experiment was performed twice with similar outcome.

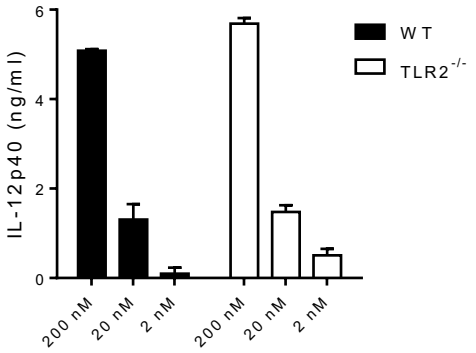


Supplementary figure 3. Activation of non-transfected HEK293 control cells as determined by levels of secreted IL-8 measured in the supernatant of stimulated cells. TNFα serves as a receptor-independent activator of HEK293 cells.

A

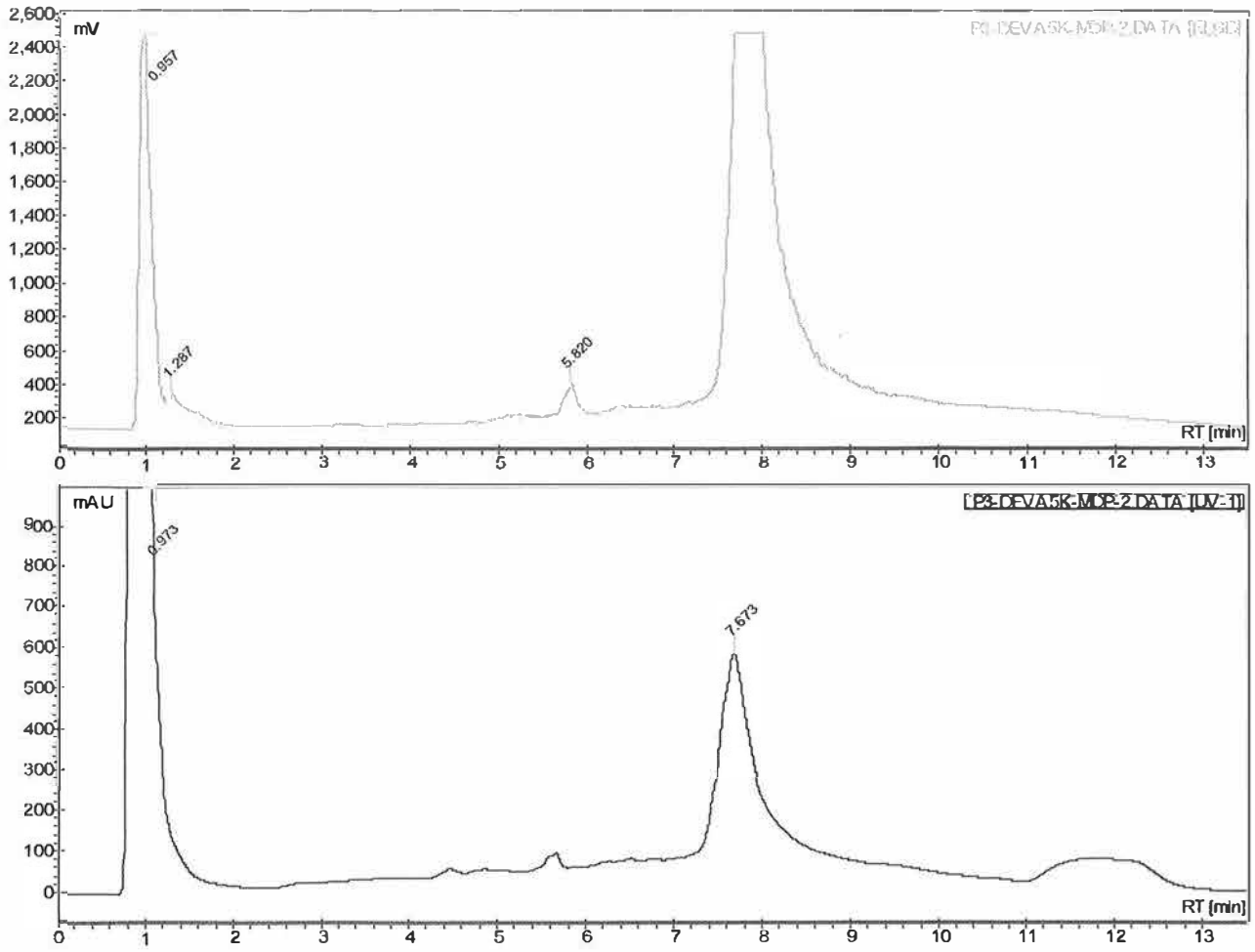


B



Supplementary figure 4. (A) Expression of activation markers CD40 and CD86 on the cell surface of WT and TLR2$^{-/-}$ BMDCs as determined by flow cytometry. MFI: geometric mean fluorescence intensity. (B) Activation of BMDCs derived from WT and TLR2$^{-/-}$ C57BL/6 mice by TLR9-L CpG, as determined by the concentration of secreted IL-12p40 in the supernatant of overnight stimulated cells. Both experiments were repeated at least twice with similar outcome.

Chromatogram : P3-DEVA5K-MDP-2_channel1



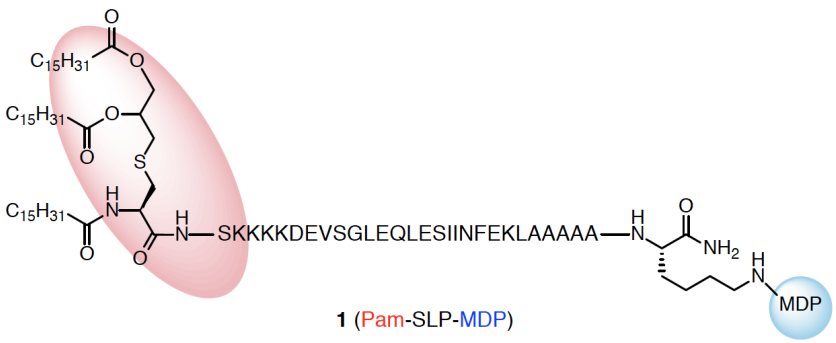
System : LCMS
Method : 1090%B10 15min
User : Users

Acquired : 11/30/2009 1:47:22 PM
Processed : 11/30/2009 2:01:20 PM
Printed : 11/30/2009 2:32:05 PM

P3-DEVA5K-MDP-2.DATA [UV-1]

Index	Time	Height	Area	Area %
	[Min]	[mAU]	[mAU.Min]	[%]
1	0.973	2776.7	847.8	76.549
2	7.673	505.3	259.7	23.451
Total		3282.0	1107.6	100.000

LC-MS UV trace , conjugate 1

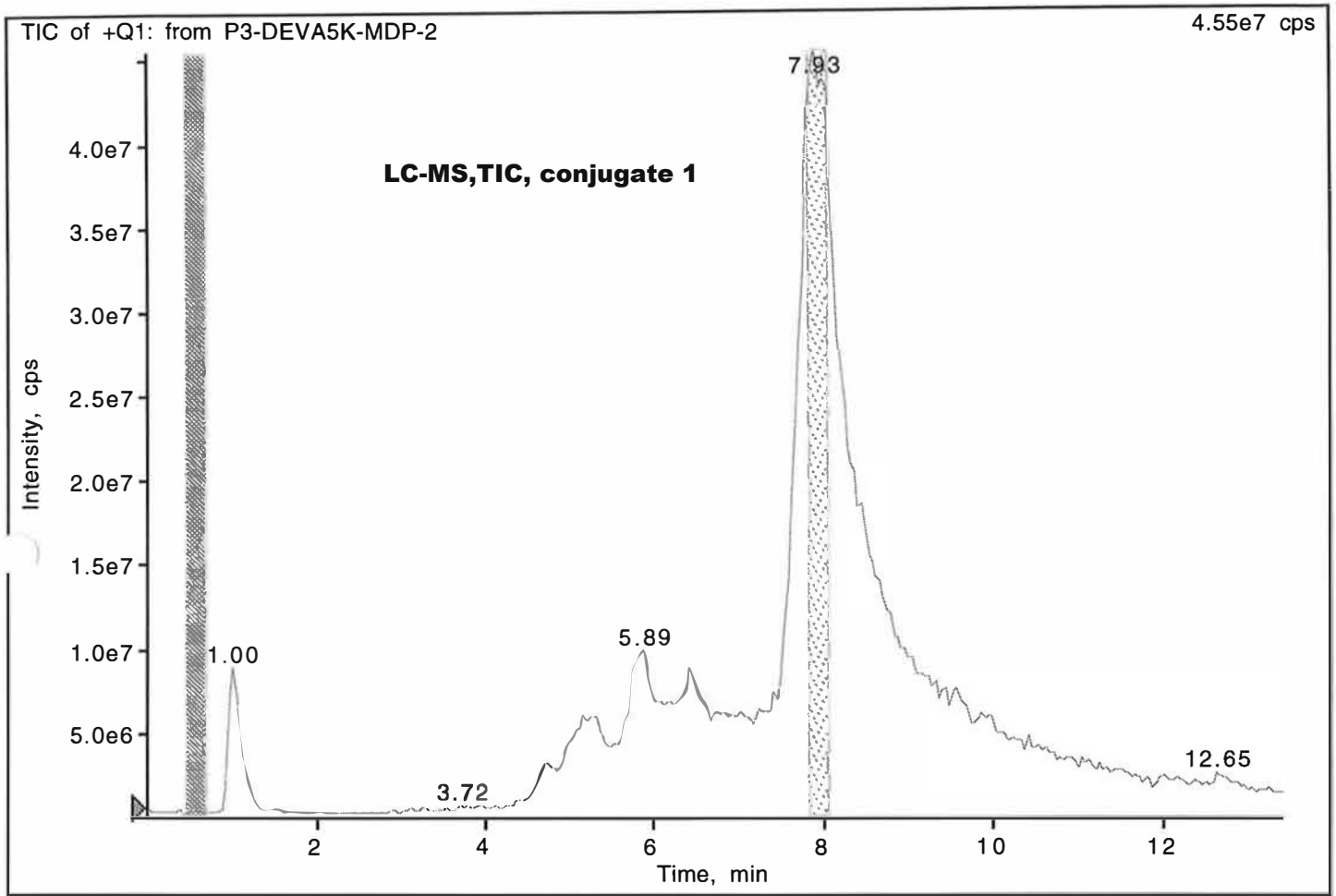


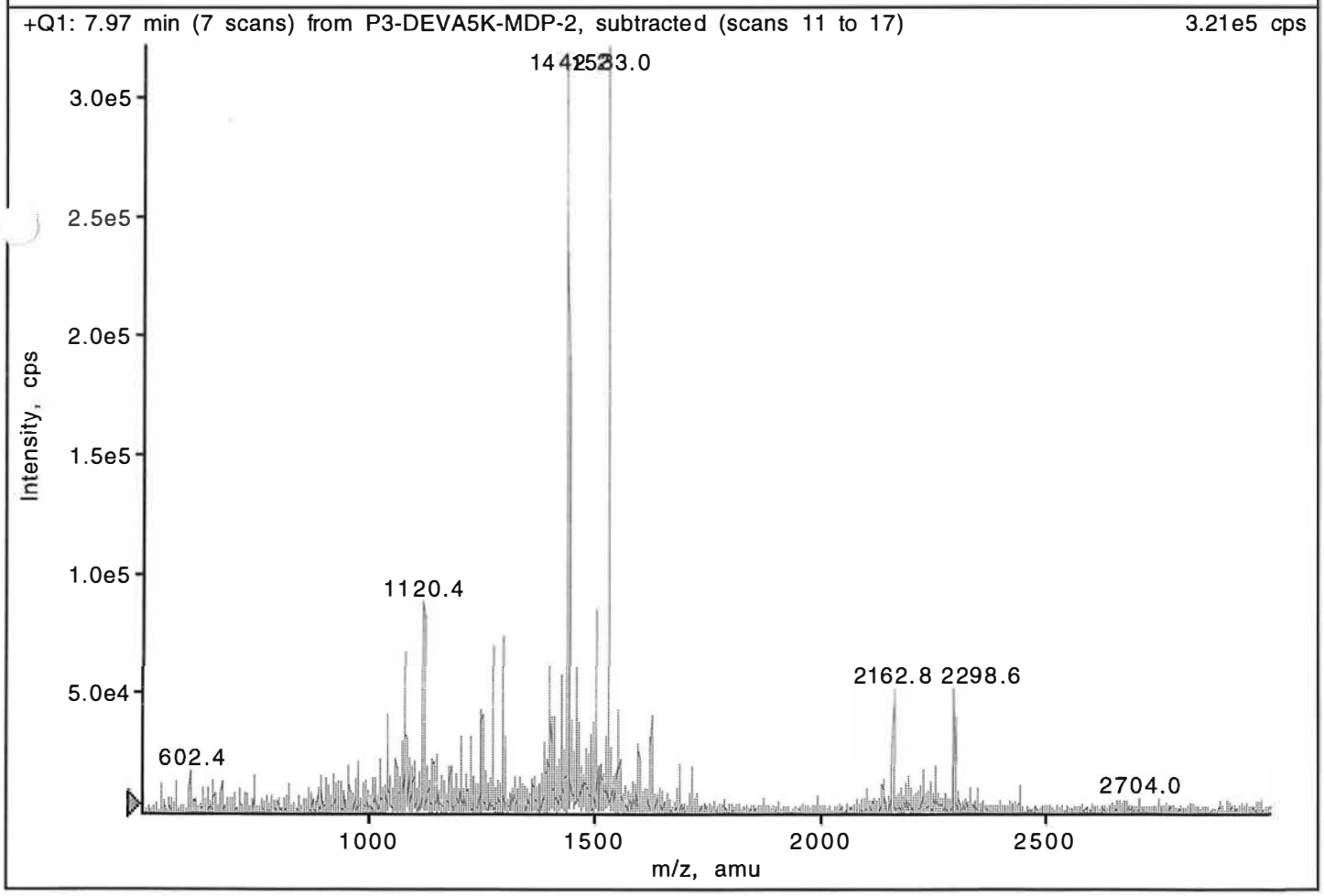
1 (Pam-SLP-MDP)

Info for pane 2: P3-DEVA5K-MDP-2

Period 1, Expt. 1; Mass range: 500.0 to 3000.0 by 0.2 amu; Dwell: 0.2 ms; Pause: 5.0 ms

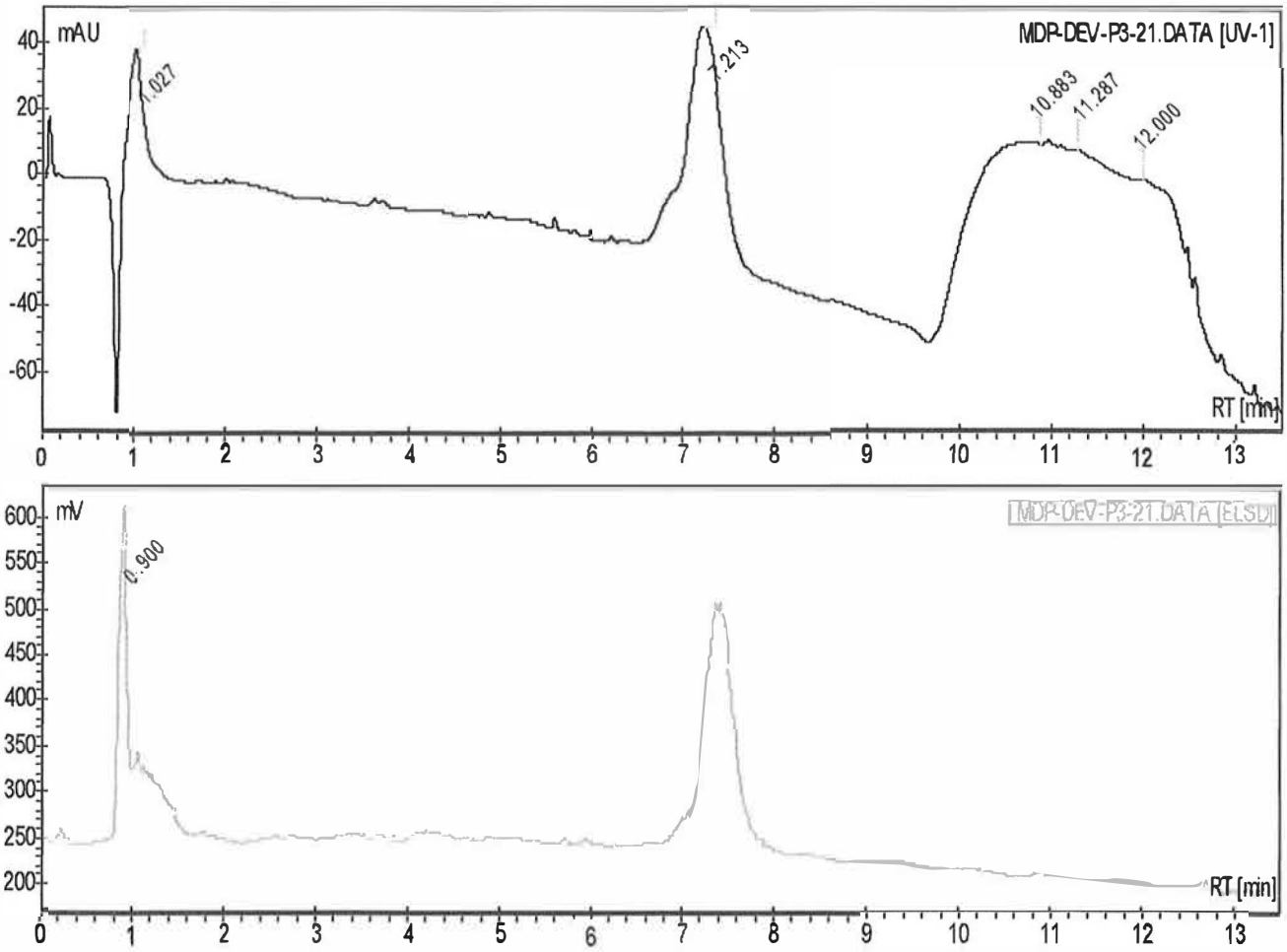
Acq. Time: maa, 30 nov 2009 at 14:55:06; Exp. Comment: Vpg analyse MM>3000 (variabele OR en RNG)





3A

Chromatogram : MDP-DEV-P3-21_channel4



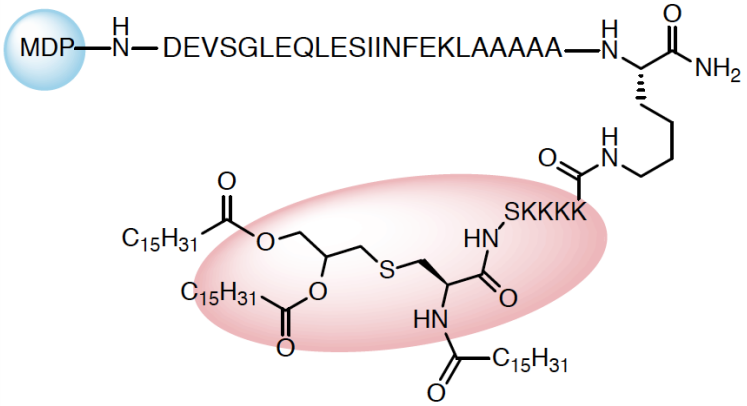
MDP-DEV-P3-21.DATA [ELSD]

Index	Time	Height	Area	Area %
	[Min]	[mV]	[mV.Min]	[%]
1	0.900	334.4	31.3	100.000
Total		334.4	31.3	100.000

System : LCMS
Method : 5090%B50 15min
User : Users

Acquired : 6/9/2011 3:20:33 PM
Processed : 6/9/2011 3:34:32 PM
Printed : 6/9/2011 4:45:09 PM

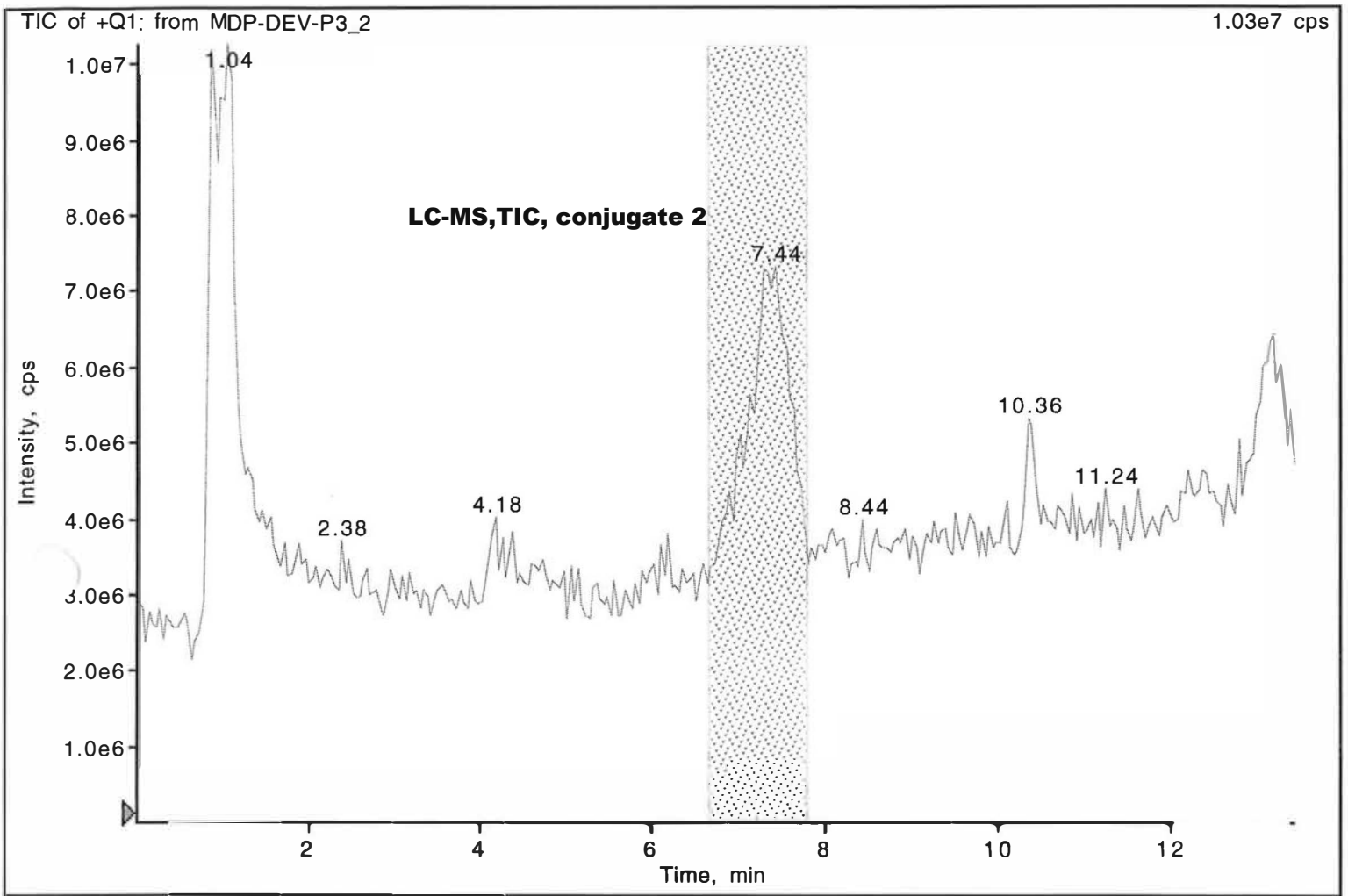
LC-MS UV-trace, conjugate 2

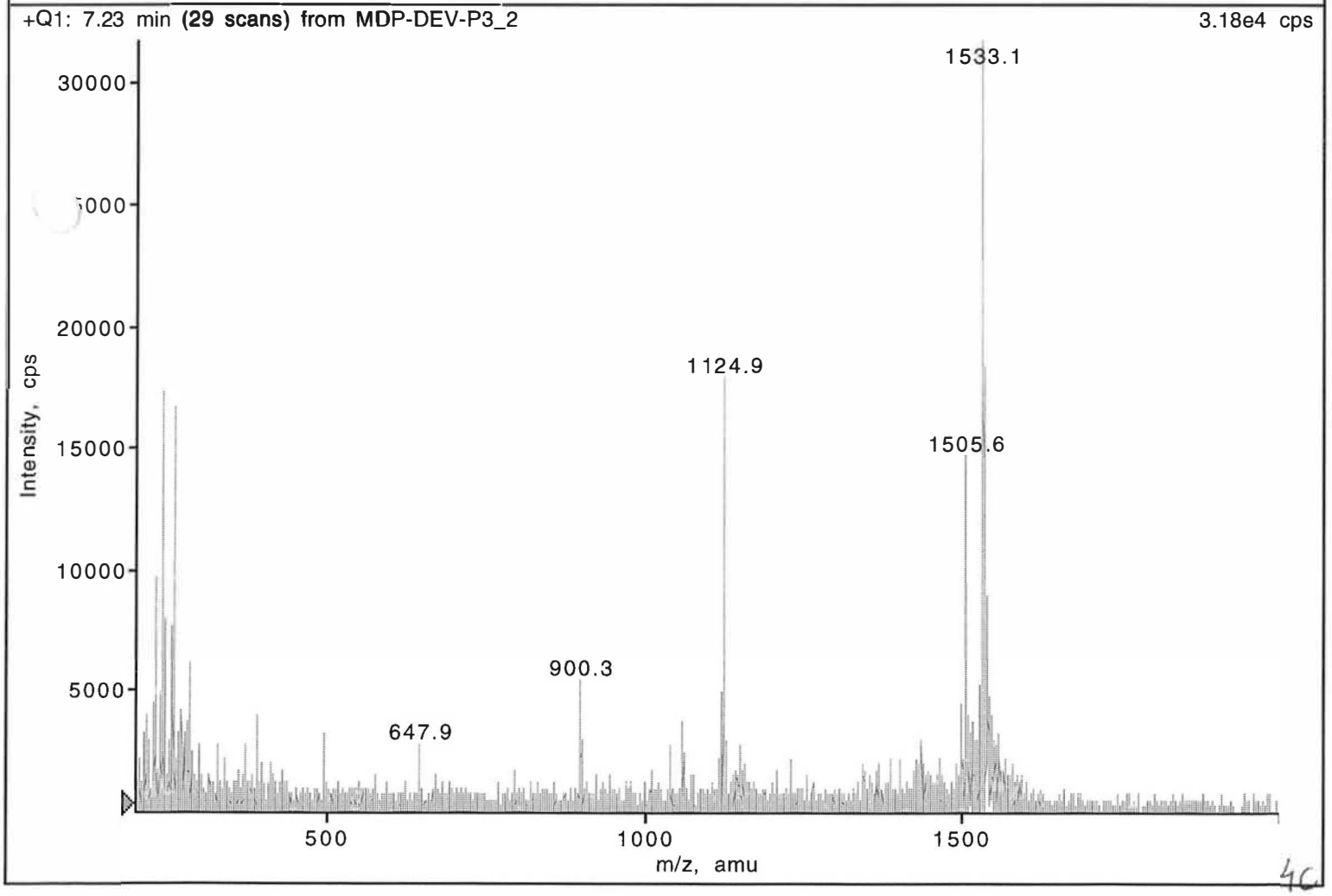


2 (MDP-SLP-Pam)

4B

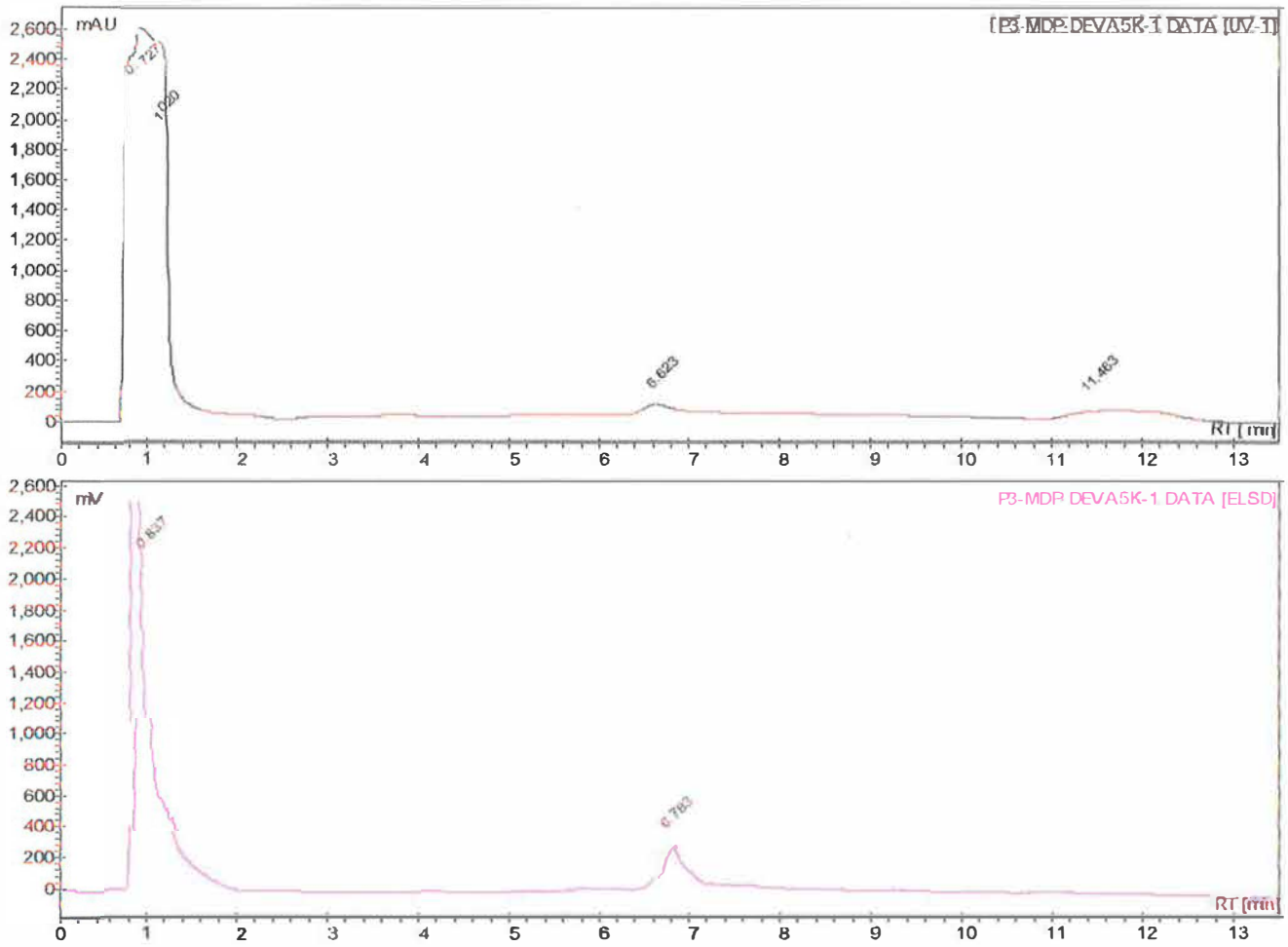
Info for pane 2: MDP-DEV-P3_2
Period 1, Expt. 1; Mass range: 200.0 to 2000.0 by 0.1 amu; Dwell: 0.1 ms; Pause: 5.0 ms
Acq. Time: don, 9 jun 2011 at 14:41:47; Exp. Comment: Default Comment.





4C

Chromatogram : P3-MDP-DEVA5K-1_channel1



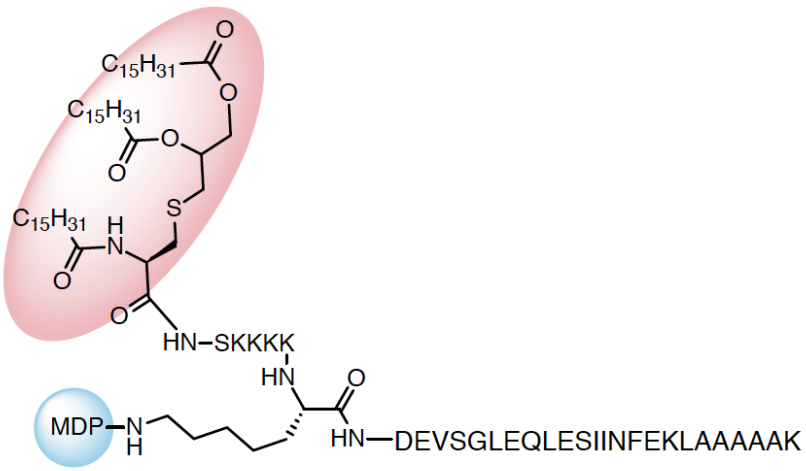
System : LCMS
Method : 1090%B10 15min
User : Users

Acquired : 9/6/2010 3:51:09 PM
Processed : 9/6/2010 4:05:05 PM
Printed : 9/6/2010 4:13:59 PM

P3-MDP-DEVA5K-1.DATA [UV-1]

Index	Time	Height	Area	Area %
	[Min]	[mAU]	[mAU.Min]	[%]
1	0.727	1905.4	457.6	87.230
2	1.020	490.8	21.7	4.140
3	6.623	65.6	24.3	4.625
4	11.463	30.8	21.0	4.005
Total		2492.6	524.6	100.000

LC-MS UV trace , conjugate 3

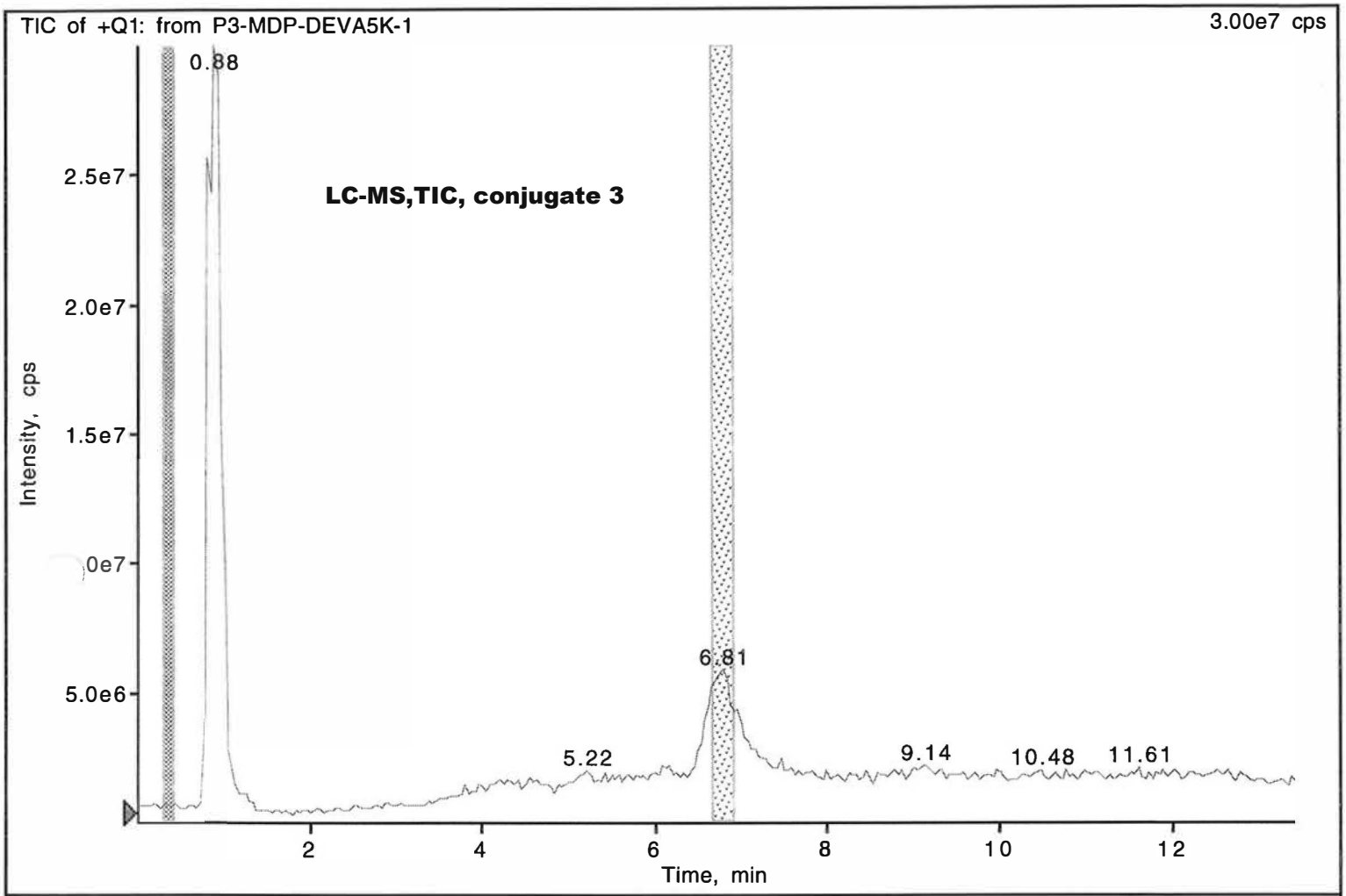


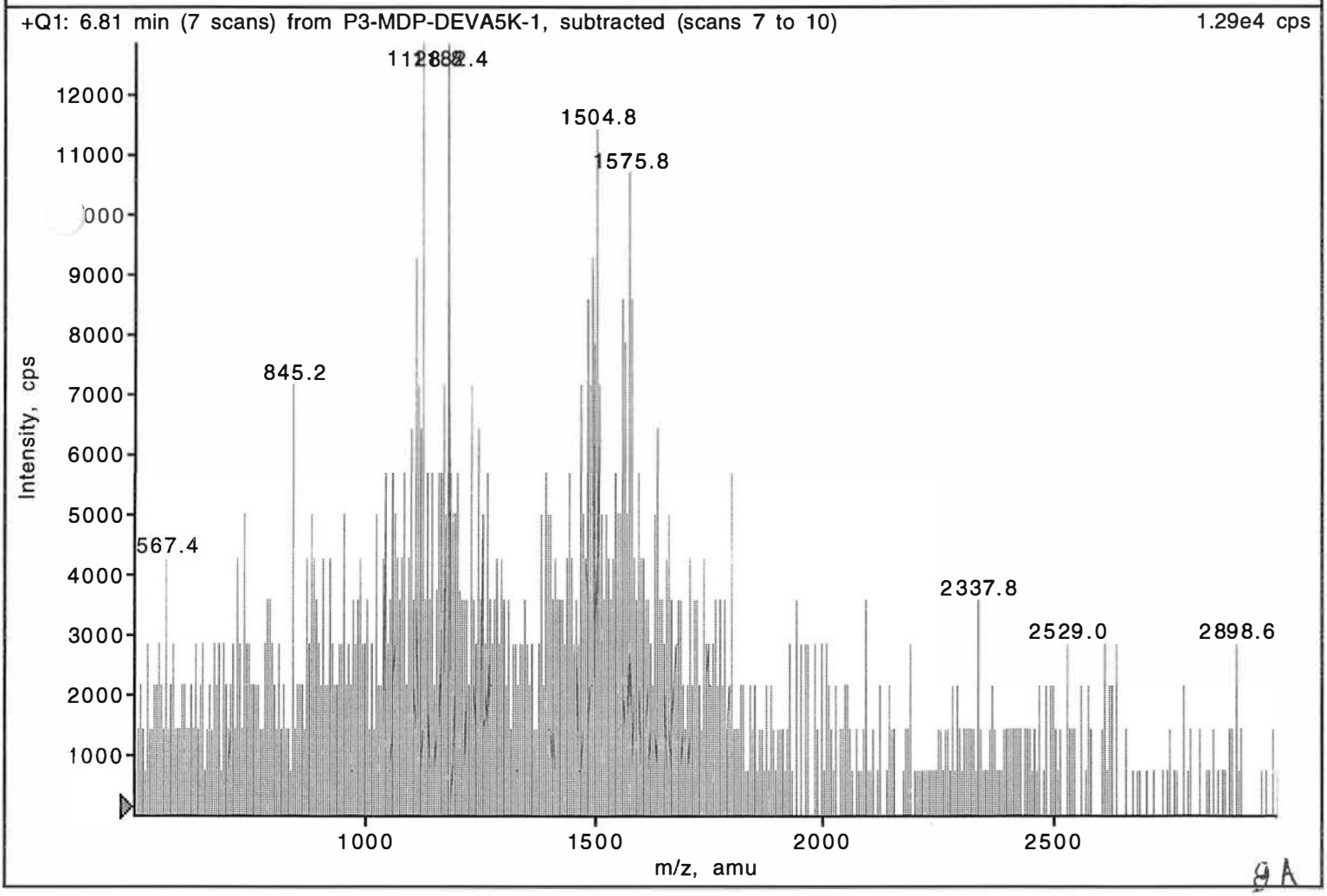
3 (Pam-MDP-SLP)

Info for pane 2: P3-MDP-DEVA5K-1

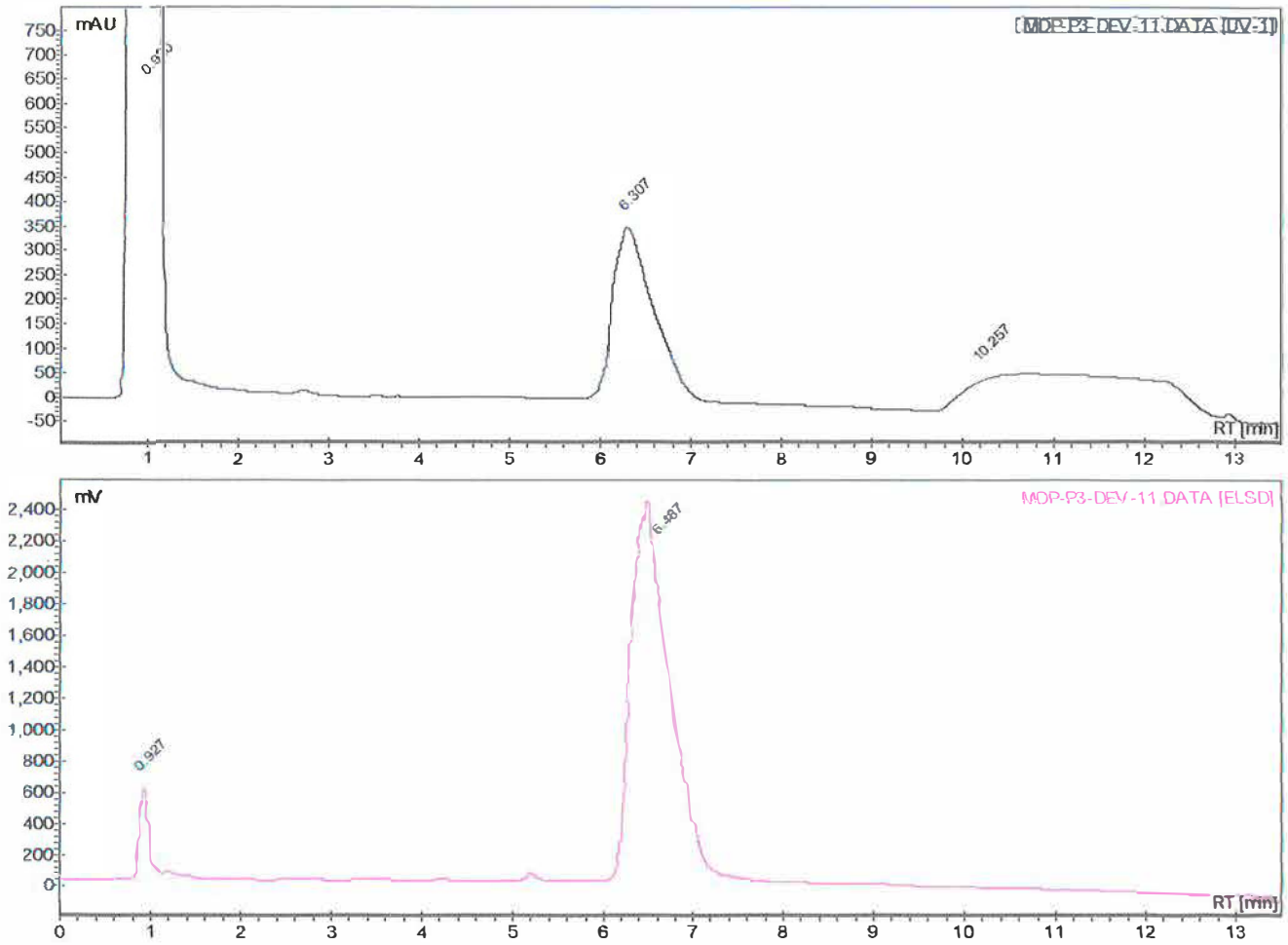
Period 1, Expt. 1; Mass range: 500.0 to 3000.0 by 0.2 amu; Dwell: 0.2 ms; Pause: 5.0 ms

Acq. Time: maa, 6 sep 2010 at 15:05:39; Exp. Comment: Vpg analyse MM>3000 (variabele OR en RNG)





Chromatogram : MDP-P3-DEV-11_channel1 C4



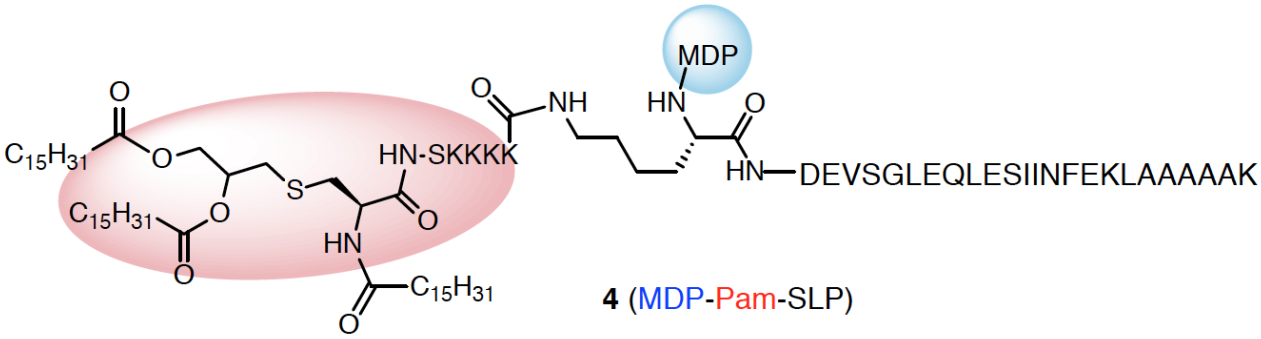
System : LCMS
Method : 5090%B50 15min
User : Users

Acquired : 9/28/2010 5:15:56 PM
Processed : 9/28/2010 5:29:54 PM
Printed : 9/29/2010 8:55:34 AM

MDP-P3-DEV-11.DATA [UV-1]

Index	Time	Height	Area	Area %
	[Min]	[mAU]	[mAU.Min]	[%]
1	0.920	2502.6	937.4	81.930
2	6.307	355.1	185.6	16.217
3	10.257	30.7	21.2	1.853
Total		2888.4	1144.2	100.000

LC-MS UV trace , conjugate 4

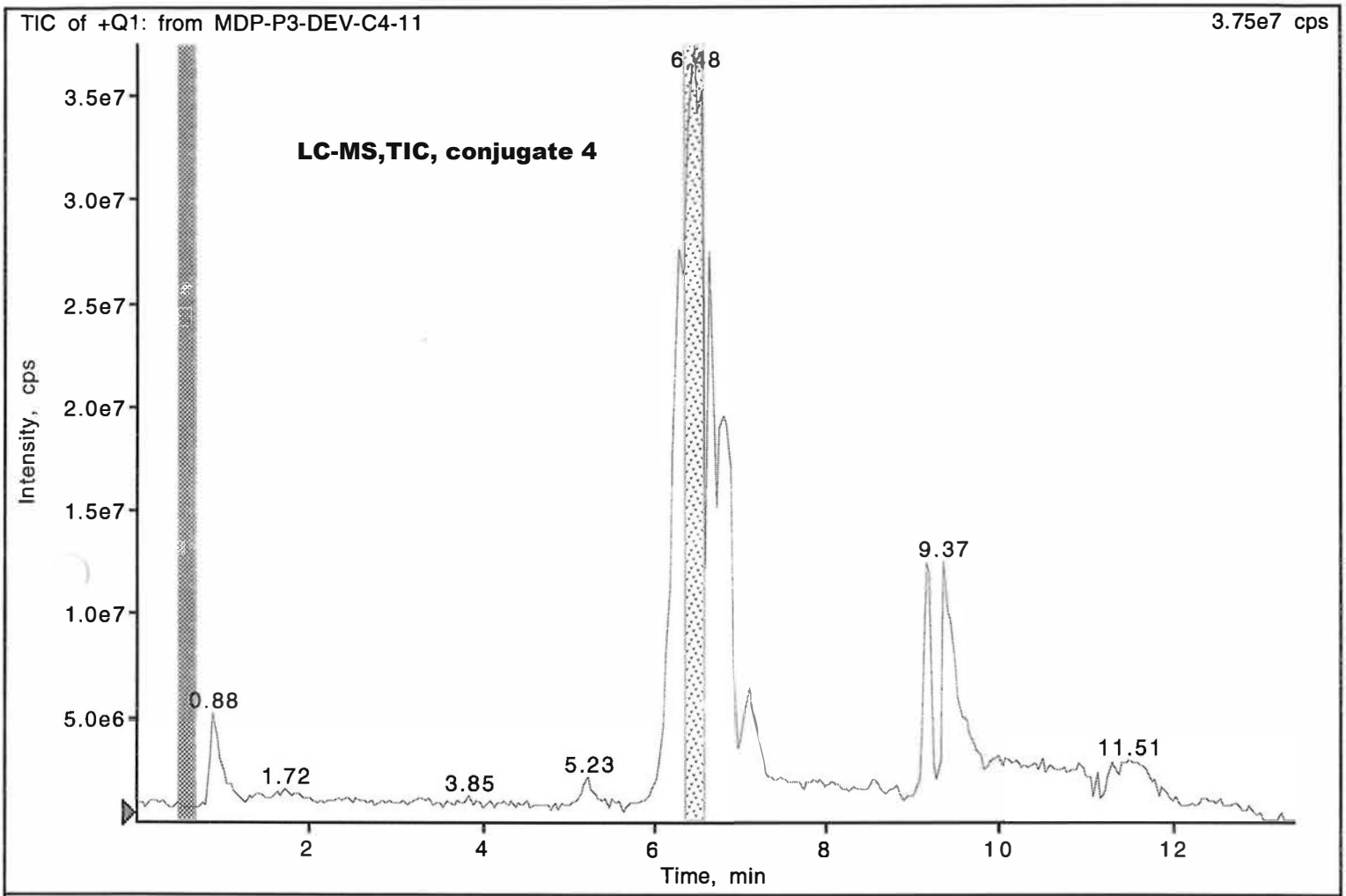


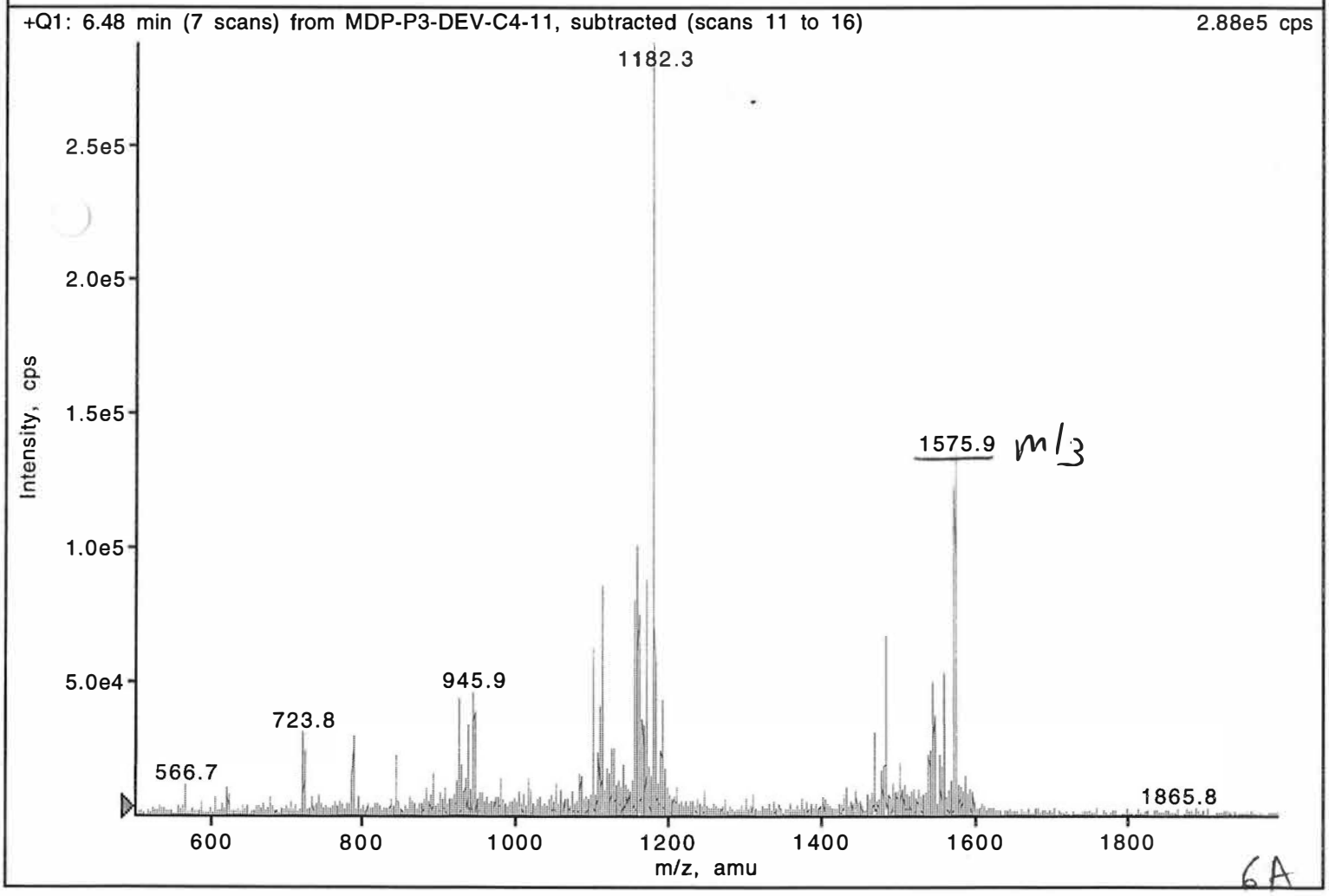
4 (MDP-Pam-SLP)

Info for pane 1: MDP-P3-DEV-C4-11

Period 1, Expt. 1; Mass range: 500.0 to 2000.0 by 0.1 amu; Dwell: 0.2 ms; Pause: 5.0 ms

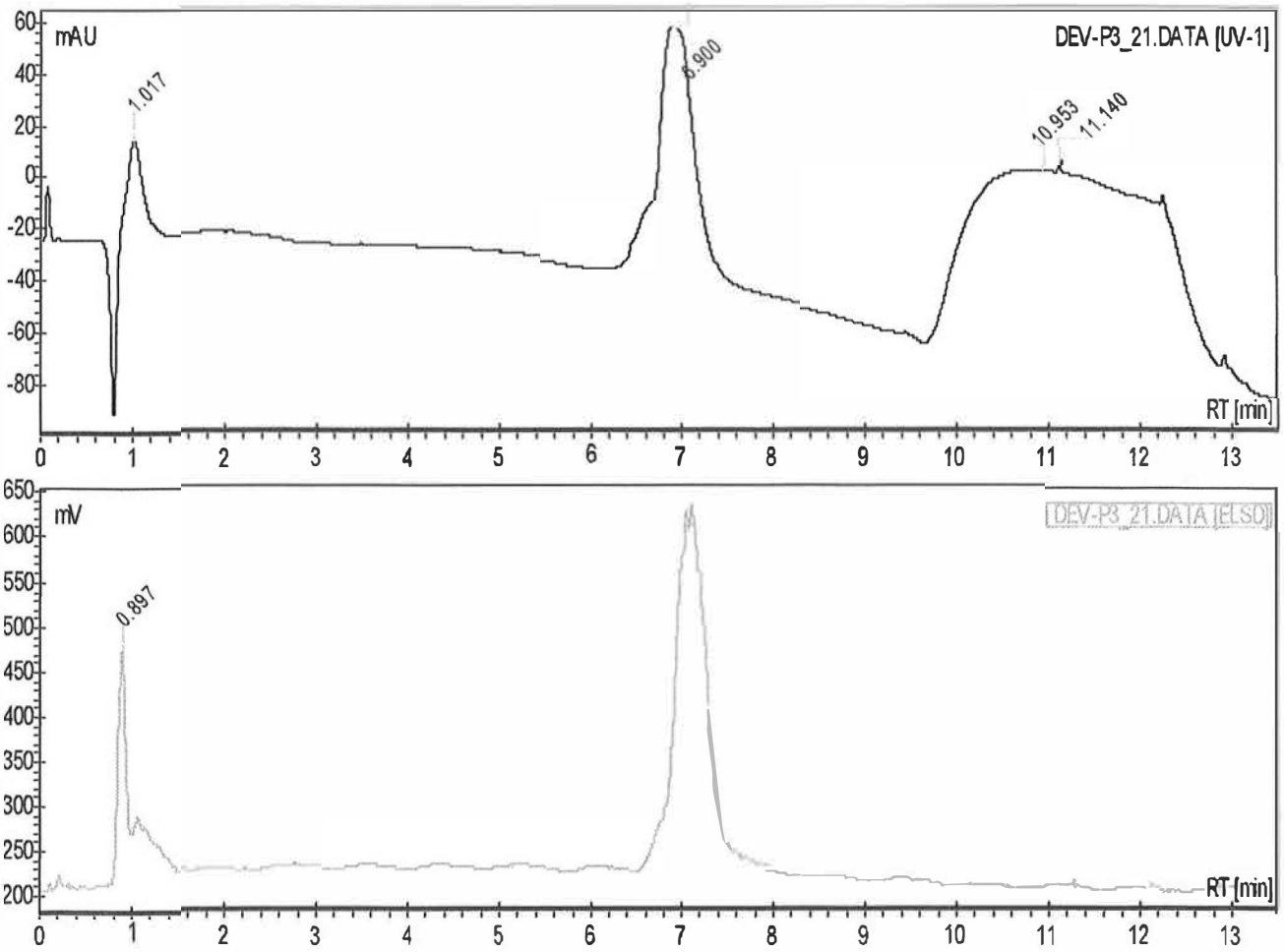
Acq. Time: din, 28 sep 2010 at 16:30:59; Exp. Comment: Default Comment.





6A

Chromatogram : DEV-P3_21_channel4



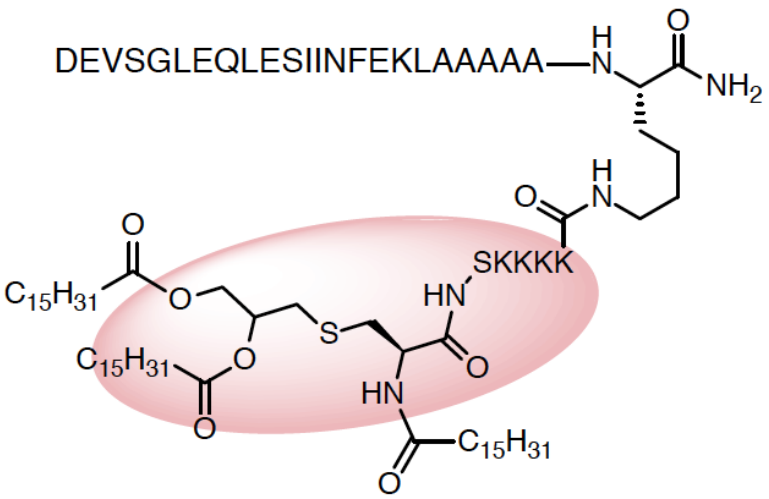
System : LCMS
Method : 5090%B50 15min
User : Users

Acquired : 6/9/2011 3:37:07 PM
Processed : 6/9/2011 3:51:09 PM
Printed : 6/9/2011 4:44:32 PM

DEV-P3_21.DATA [ELSD]

Index	Time	Height	Area	Area %
	[Min]	[mV]	[mV.Min]	[%]
1	0.897	238.0	21.9	100.000
Total		238.0	21.9	100.000

LC-MS UV trace , conjugate 2S

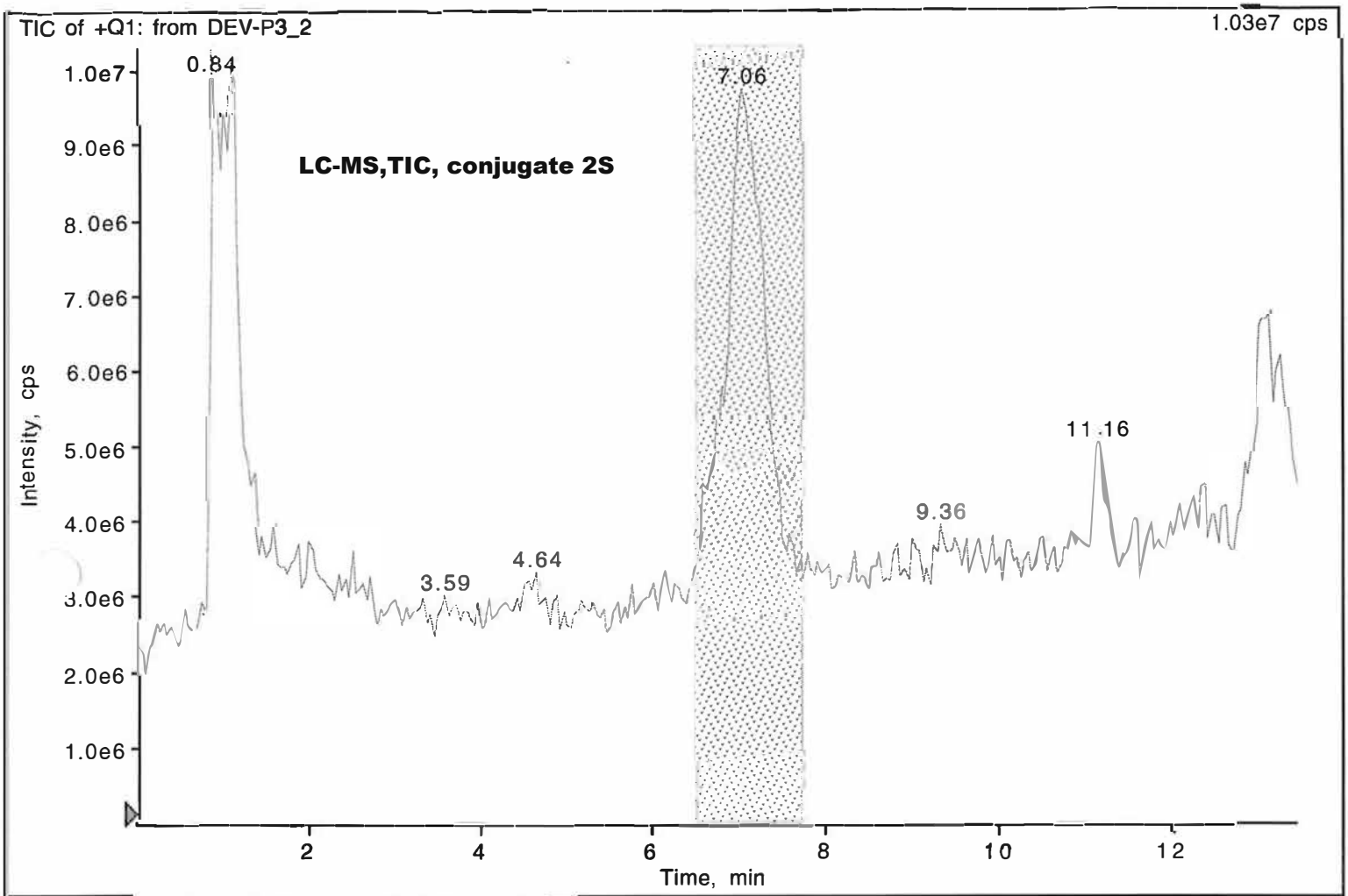


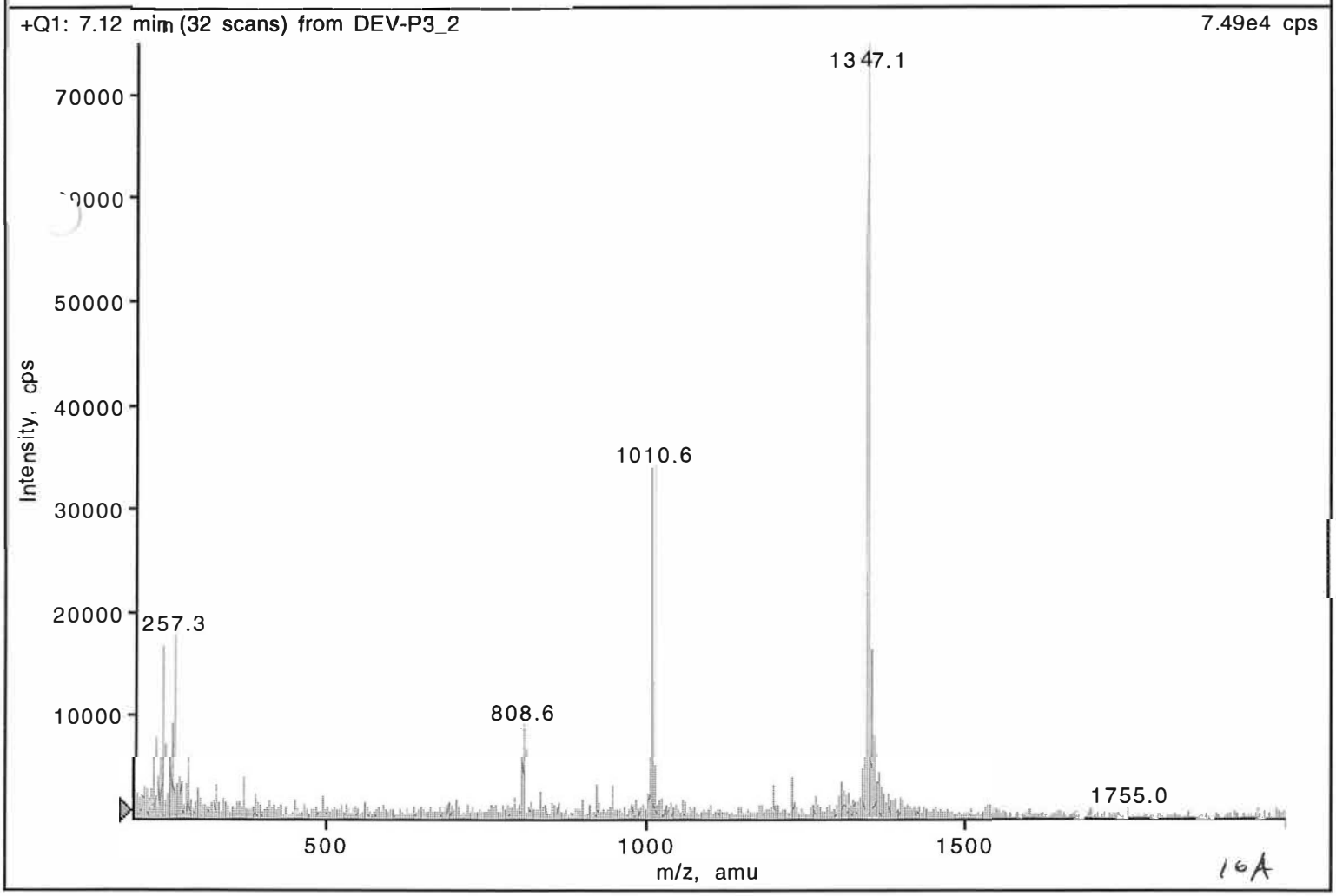
2S (SLP-Pam)

Info for pane 2: DEV-P3_2

Period 1, Expt. 1; Mass range: 200.0 to 2000.0 by 0.1 amu; Dwell: 0.1 ms; Pause: 5.0 ms

Acq. Time: don, 9 jun 2011 at 14:58:21; Exp. Comment: Default Comment.





16A