CHAPTER IV
RESULTS AND DISCUSSION

This research was related to the development of mass spectrometric method for quantitative determination of alkylamine ethoxylate (surfactant). As proposed in previous chapter, the results will be shown and discussed in each part, respectively.

4.1 The optimized conditions and parameters for determination of molecular weight distribution of alkylamine ethoxylate.

4.1.1 Target preparation

For MALDI-MS analysis, a comparison of MALDI spectra of surfactants from a dried droplet and thin layer method was investigated. The results in Figure A1 showed that the dried droplet method gave higher intensity of sample peaks in mass spectrum than thin layer method. This may be because the target which prepared from dried droplet method is more homogeneous than the thin layer preparation method.

4.1.2 Types of matrices and laser power

Types of matrices and laser power are the most significant factors for MALDI analysis because the matrix and laser power corresponded to ionization efficiency of the sample. Moreover, the matrices yield the difference in range of molecular weight distribution, laser power and noise. The matrices: *all*-trans retinoic cid (RTA), dithranol, 2-(4-hydroxyphenylazo)-benzoic acid (HABA), indole acrylic acid (IAA), 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CCA), were used in the experiment.

Types of matrices yield a difference of m/z range of molecular weight distribution. The spectra were shown in Figure A2 and summarized in Table 4.1.

Table 4.1 Mass to charge molecular weight distribution and laser power of matrices.

Type of matrix	Laser power (μJ)	m/z range of molecular weight distribution
all-trans retinoic cid	320	400-800, 600-1200
dithranol	360	600-1200
2-(4.hydroxyphenylazo)-benzoic acid	320	400-1200
indole acrylic acid	300	400-1200
2,5-dihydroxybenzoic acid	360	400-1200
α-cyano-4-hydroxycinnamic acid	260	400-1200

All-trans retinoic acid (RTA)

From Figure A2 (a), there are two m/z ranges of molecular weight distribution in the range of m/z of 400-800 and 600-1200. The repeating unit of this polymer is 44 Da which is obtained at laser power value of 320 μJ. The high intensity of noise is also obtained. Thus, this matrix is not suitable for determination of molecular weight distribution of alkylamine ethoxylate. The repeating unit at 44 Da is corresponding to the repeating unit of alkylamine ethoxylate.

Dithranol

Although there is only one m/z range of molecular weight distribution in m/z of 600-1200, with a repeating unit of 44 Da. The intensity of major and minor series of carbon chain of surfactant are different clearly by using dithranol as the matrix at laser power value of 360 μJ are shown in Figure A2 (b), which are pleasant results but the intensity of noise is high in m/z of 400-700. Hence, the matrix is not proper matrix for determination of molecular weight distribution of alkylamine ethoxylate.

2-(4-Hydroxyphenylazo)-benzoic acid (HABA)

The MALDI-MS spectrum of alkylamine ethoxylate with HABA as the matrix at laser power 320 μJ is shown in Figure A2 (c). Molecular weight distribution is in a mass range of m/z of 400-1200, with a repeating unit of 44 Da. The intensity of noise is high which is the same as when use dithranol as a matrix. Hence, this matrix is not suitable for determination of molecular weight distribution of alkylamine ethoxylate.

Indole acrylic acid (IAA)

The MALDI-MS spectrum of alkylamine ethoxylate with IAA as the matrix at laser power value of 300 μJ is shown in Figure A2 (d). Molecular weight distribution is in the range of m/z of 400-1200, with a repeating unit of 44 Da. The intensity of noise is similar to using dithranol and HABA as matrices. Then, this matrix is not proper for determination of molecular weight distribution of alkylamine ethoxylate.

2,5-Dihydroxybenzoic acid (DHB)

This matrix is very appropriate to determination of molecular weight distribution of alkylamine ethoxylate because the spectrum from Figure A2 (e) showed only one m/z range of molecular weight distribution in 400-1200, with a repeating unit of 44 Da. The intensity of major and minor series of carbon chain of surfactant is clearly different when laser power of 360 μJ was used. Although, DHB gave the proper results for using as matrix to qualitative analysis of alkylamine ethoxylate but DHB was not chosen to be a matrix for analysis of alkylamine ethoxylate in this research because DHB cannot make a good signal when it was used as a matrix for neurotensin which was used as internal standard in quantitative analysis of alkylamine ethoxylate.

α-cyano-4-hydroxycinnamic acid (CCA)

The MALDI-MS spectrum of alkylamine ethoxylate with CCA as a matrix at laser power of 260 μJ is shown in Figure A2 (f). Molecular weight distribution is in the range of a m/z of 400-1200, with a repeating unit 44 Da. Although, the intensity of major and minor series of carbon chain of alkylamine ethoxylate is not much different like using DHB as the matrix whereas the signal of neurotensin with CCA as a matrix can occur very well. CCA was chosen to be the matrix for analysis of alkylamine ethoxylate.

4.1.3 Precision

The precision of instrument was tested by loading sample to the target with the same amount repeat in various positions. The 1.0 μL mixture of analyte and matrix was dropped on target with several times. Then the 0.5 μL mixture was dropped on target with several times. The results were shown in Table 4.2.The mass spectra when used 1.0 μL mixture for the analysis were shown in Figure A3 and the mass spectra when used 0.5 μL mixture for the analysis were shown in Figure A4.

Table 4.2 Approximate intensities of 1.0 μL and 0.5 μL

Time	Approximate Intensity of 1.0 μL (a.i.)	Approximate Intensity of 0.5 μL (a.i.)
1	10,000	8,000
2	10,500	5,000
3	26,000	7,500
4	25,000	7,500
5	5,000	7,500
6	15,000	7,000
7	7,000	8,000
8	10,000	9,000

From Table 4.2, dropping with 0.5 μl gave more precise than dropping with 1.0 μl because the mixtures which were dropped of 0.5 μL is more homogeneous than those of 1.0 μL. And then, the intensities were occurred precisely.

4.1.4 Ratios of matrix to analyte

The same concentration of alkylamine ethoxylate was mixed with the matrix solution (CCA) with a various ratios of 1:140, 1:160, 1:180, 1:200, 1:220, 1:240, 1:260 and

1:280. The spectra were shown in Figure A5. The average intensity from each spectrum was shown in Table 4.3.

Table 4.3. Average intensity of ratios of analyte to matrix of various ratios of analyte-to-matrix of alkylamine ethoxylate

Ratio of analyte to matrix	Average intensity (a.i.)
1:2140	2040
1:160	4346
1:180	4742
1:200	7023
1:220	9156
1:240	7739
1:260	2836
1:280	2107

Table 4.3 suggested that when ratio of matrix was increased the average intensity of signal be increased rapidly. Until the ratio of analyte to matrix reached to 1:220 then the average intensity of signal began decrease. The relationship between ratios of analyte to matrix and average intensities was shown in Figure 4.1. Ratio of analyte to matrix which gave the highest average intensity was 1:225. This ratio is the most suitable for quantitative analysis.

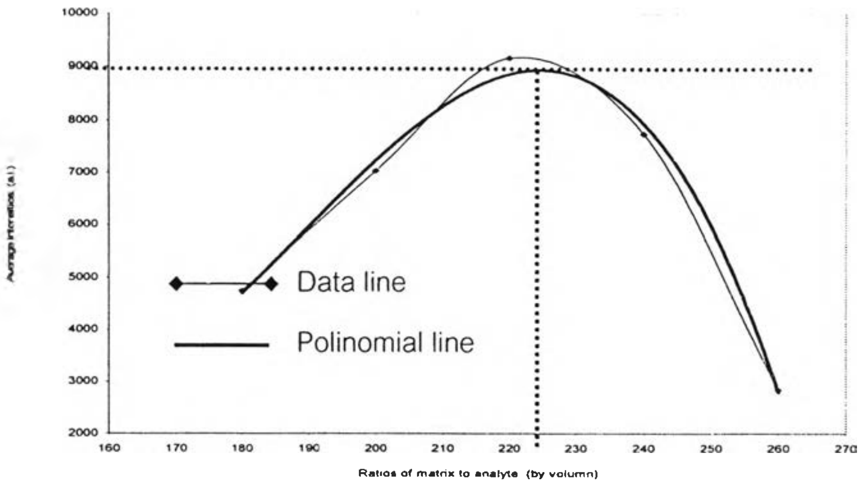


Figure 4.1 Plot of ratios of analyte to matrix versus average intensities.

4.2 Determination of molecular weights of surfactant polymers

Mass-average molecular weight and number-average molecular weight are calculated using the following equation.

1) Mass –average molecular weight; $\overline{Mw}$

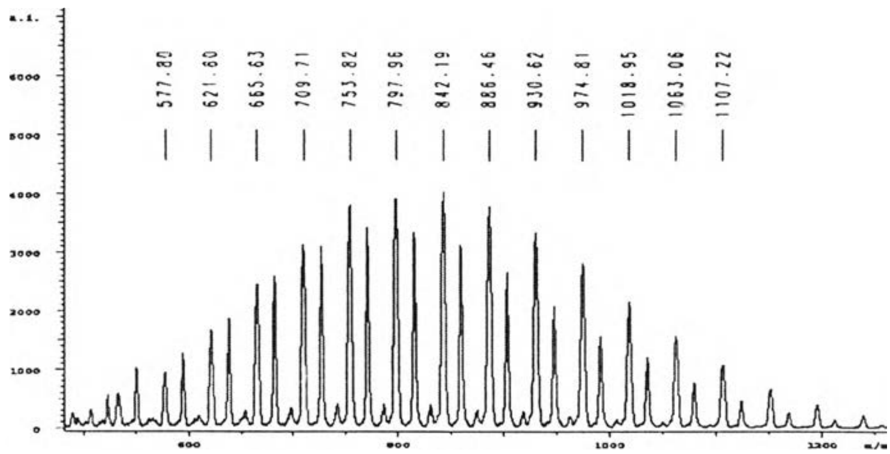
$$\overline{Mw} = \frac{\sum(N_i M_i^2)}{\sum(N_i M_i)} \tag{4.1}$$

2) Number-average molecular weight; $\overline{Mn}$

$$\overline{Mn} = \frac{\sum(N_i M_i)}{\sum N_i} \tag{4.2}$$

Where N_i, M_i represent signal intensity in peak area and mass for the oligomer containing *i* monomer

Example 4.1



$$\overline{Mw} = \frac{\sum(N_i M_i^2)}{\sum(N_i M_i)}$$

= [(0.0334X577.8^2) + (0.0588X621.60^2) + (0.0864X655.63^2) + (0.1099X70971^2) + (0.1338X753.82^2) + 0.1374X797.96^2) + (0.1410X842.19^2) + (0.1325X886.46^2) + (0.1169X930.62^2) + (0.0986X974.81^2) + (0.0756X1018.95^2) + (0.0551X1063.06^2) + (0.0374X1107.22^2)] / [(0.0334X577.8) + (0.0588X621.60) + (0.0864X655.63) + (0.1099X70971) + (0.1338X753.82) + 0.1374X797.96) + (0.1410X842.19) + (0.1325X886.46) + (0.1169X930.62) + (0.0986X974.81) + (0.0756X1018.95) + (0.0551X1063.06) + (0.0374X1107.22)]

= 859.3437

$$\overline{Mn} = \frac{\sum(N_i M_i)}{\sum N_i}$$

= [(0.0334X577.8) + (0.0588X621.60) + (0.0864X655.63) + (0.1099X70971) + (0.1338X753.82) + 0.1374X797.96) + (0.1410X842.19) + (0.1325X886.46) + (0.1169X930.62) + (0.0986X974.81) + (0.0756X1018.95) + (0.0551X1063.06) + (0.0374X1107.22)] / (0.0334+0.0588+0.0864+0.1099+0.1338+0.1374+0.1410+0.1325+0.1169+0.0986+0.0756+0.0551+0.0374)

= 837.7028

$D_p = \dfrac{\overline{M_w}}{\overline{M_n}}$ = 859.6437/837.7028 = 1.0258

$\overline{Mw}$, $\overline{Mn}$ of alkylamine ethoxylate standard and surfactants in herbicides were calculated average values and were shown in Table 4.4.

Table 4.4a $\overline{Mw}$, $\overline{Mn}$ and D_p values of alkylamine ethoxylate and surfactants in herbicides

Alkylamine ethoxylate standard (TERWET 3780)			Surfactant in commercial-A			Surfactant in commercial-B		
$\overline{Mw}$	$\overline{Mn}$	D_p	$\overline{Mw}$	$\overline{Mn}$	D_p	$\overline{Mw}$	$\overline{Mn}$	D_p
886.41	850.38	1.04	858.3	842.12	1.02	851.66	833.66	1.02
867.96	852.23	1.02	791.27	771.98	1.02	848.55	830.60	1.02
866.65	851.67	1.02	825.27	807.81	1.02	846.84	829.31	1.02
871.29	855.01	1.02	845.11	828.26	1.02	P54.50	837.06	1.02
859.71	842.45	1.02	828.88	814.66	1.02	851.53	833.66	1.02
869.27	856.11	1.02	826.27	809.02	1.02	858.24	841.31	1.02
Average *870.22*	*851.32*	*1.02*	*829.18*	*812.31*	*1.02*	*851.80*	*834.27*	*1.02*
Standard deviation *8.86*	*4.82*	*0.008*	*22.64*	*23.69*	*0*	*4.11*	*4.39*	*0*

Table 4.4(continue) $\overline{Mw}$, $\overline{Mn}$ and D_p values of alkylamine ethoxylate and surfactants in herbicides.

Alkylamine ethoxylate standard (RP-11)			Surfactant in commercial-C			Surfactant in commercial-D		
$\overline{Mw}$	$\overline{Mn}$	D_p	$\overline{Mw}$	$\overline{Mn}$	D_p	$\overline{Mw}$	$\overline{Mn}$	D_p
913.11	889.53	1.03	894.19	873.16	1.02	902.49	881.93	1.02
887.36	866.50	1.02	892.21	871.47	1.02	907.47	886.63	1.02
882.86	860.83	1.03	886.47	865.59	1.02	913.34	891.91	1.02
895.41	870.60	1.03	890.50	871.20	1.02	925.84	902.61	1.03
907.23	886.80	1.02	884.09	863.04	1.02	905.66	885.51	1.02
901.05	878.99	1.03	878.71	855.59	1.03	920.50	891.19	1.02
Average *897.84*	*875.54*	*1.02*	*889.21*	*866.68*	*1.02*	*912.56*	*891.20*	*1.02*
Standard deviation *11.59*	*11.46*	*0.005*	*3.43*	*6.09*	*0.004*	*9.12*	*8.05*	*0.004*

Alkylamine ethoxylate consists of the combination of hydrocarbon chains and nitrogen which bonded with a repeating unit (44 Da) of ethylene oxide ($-CH_2CH_2O-$).

$$CH_3(CH_2)_{m-1}N\begin{array}{l}(CH_2CH_2O)_xH \\ \\ (CH_2CH_2O)_yH\end{array}$$

$$R + 14 + [44(x+y) + 2] \quad = \quad M \qquad (4.3)$$

Where R is the alkyl group such as $C_{18}H_{37}$ or $C_{16}H_{33}$, x+y is the number of repeating unit and M is the molecular weight.

Alkylamine ethoxylate standard

TERWET 3780

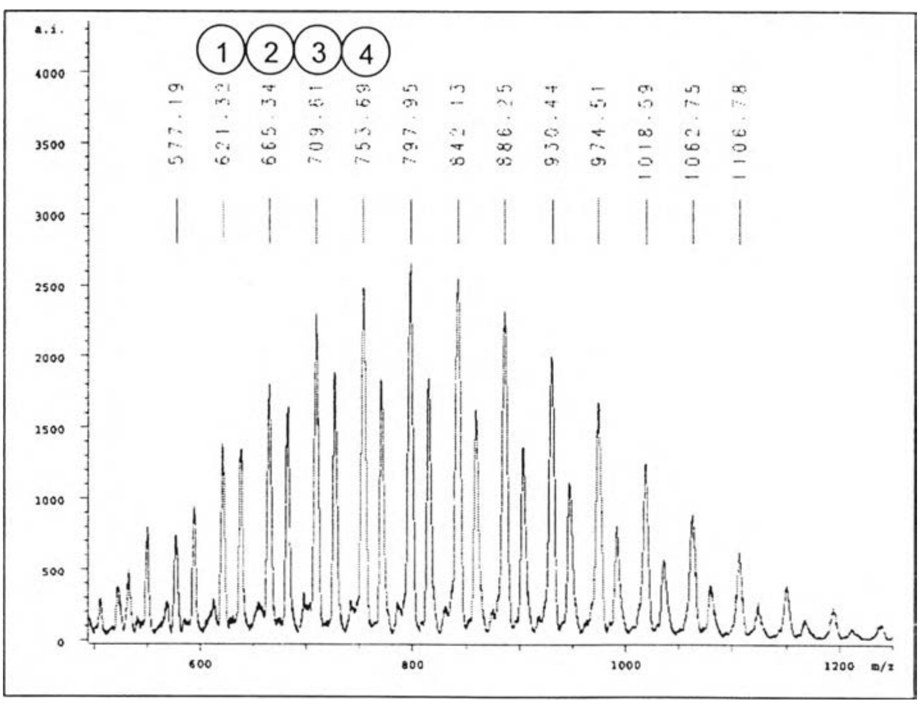


Figure 4.2 MALDI-MS spectrum of alkylamine ethoxylate standard (TERWET 3780) shown m/z of C_{18}-series.

$$R + 14 + [44(x+y) + 2] = M$$

Example 4.2

From 1 $\quad M + 1 = 621.32$

$M = 620.32$

$R + 44(x+y) = 604.32$

If $R = C_{18}H_{37}$; $\quad 44(x+y) = 351.32$

$x+y = 7.98 \sim 8$

Thus, 1 is $C_{18}H_{37}N(CH_2CH_2O)_8H_2$.

Consequently, 2 is $C_{18}H_{37}N(CH_2CH_2O)_9H_2$, 3 is $C_{18}H_{37}N(CH_2CH_2O)_{10}H_2$,

4 is $C_{18}H_{37}N(CH_2CH_2O)_{11}H_2$.

There are peaks between $C_{18}H_{37}N(CH_2CH_2O)_{x+y}$ H_2 and $C_{18}H_{37}N(CH_2CH_2O)_{x+y+1}H_2$ was shown in Figure 4.3.

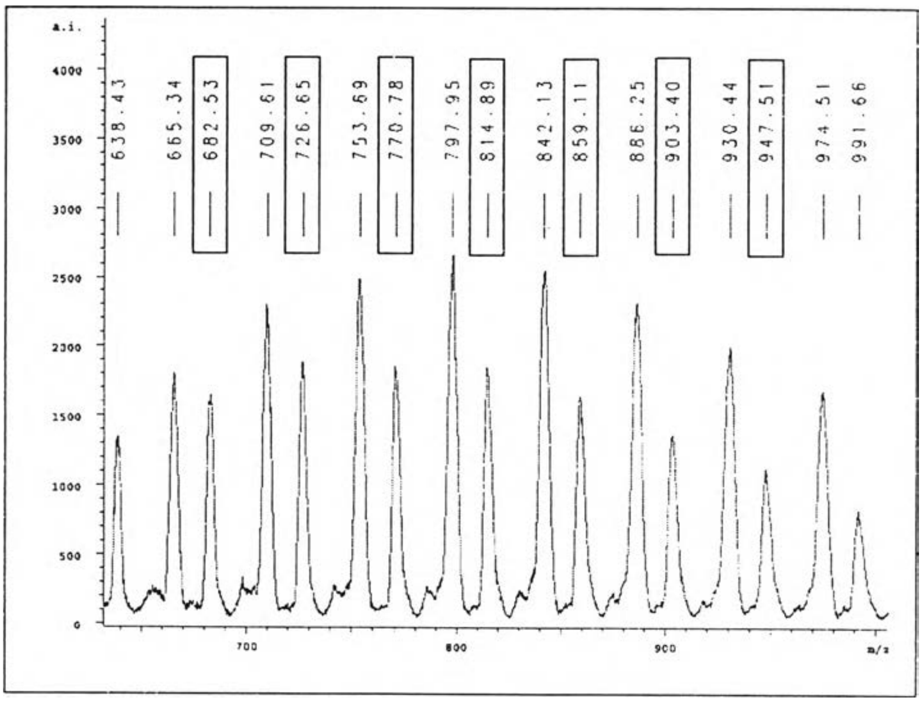


Figure 4.3 MALDI-MS spectrum of alkylamine ethoxylate standard (TERWET 3780) shown C_{16}-series.

Example 4.3

from eq. 4.3; $\quad M + 1 = 682.53$

$M = 681.53$

	$R + 44(x+y)$	=	665.53	
If $R = C_{16}H_{33}$;	$44(x+y)$	=	440.53	
	$x+y$	=	10.01	~ 10

The peak at m/z of 682.53 corresponds to $C_{16}H_{33}N(CH_2CH_2O)_{10}H_2$. Consequently, at m/z of 726.65, 770.78, 814.89, and 859.11 correspond to $C_{16}H_{33}N(CH_2CH_2O)_{11}H_2$, $C_{16}H_{33}N(CH_2CH_2O)_{12}H_2$, $C_{16}H_{33}N(CH_2CH_2O)_{13}H_2$ and $C_{16}H_{33}N(CH_2CH_2O)_{14}H$, respectively.

RPII

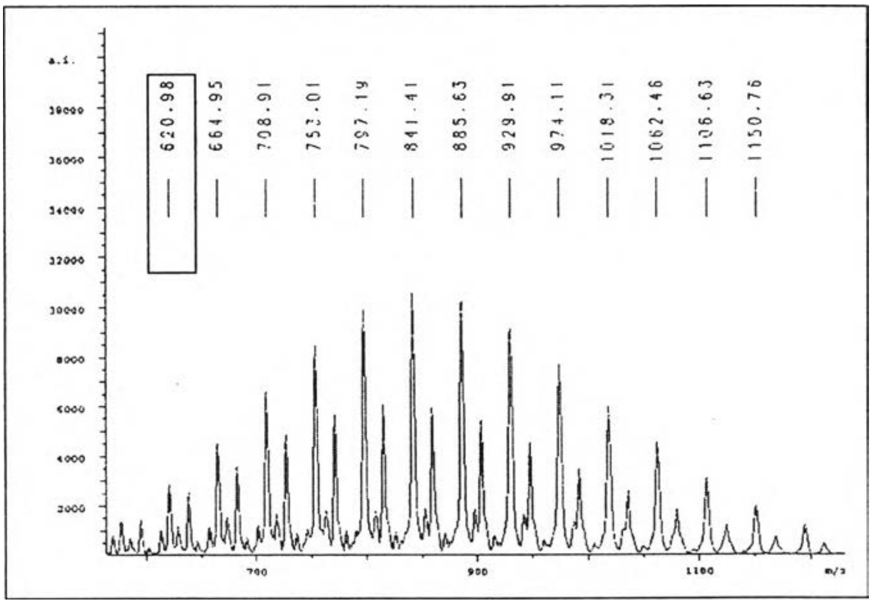


Figure 4.4 MALDI-MS spectrum of alkylamine ethoxylate standard (RPII) shown m/z of C_{18}-series.

The interpretation of Figure 4.4 uses the same calculation as example 4.2. The peak at m/z of 620.98 corresponds to $C_{18}H_{37}N(CH_2CH_2O)_8H_2$. Consequently, at m/z of 664.95, 708.91, 753.01 and 797.19 correspond to $C_{18}H_{37}N(CH_2CH_2O)_9H_2$, $C_{18}H_{37}N(CH_2CH_2O)_{10}H_2$, $C_{18}H_{37}N(CH_2CH_2O)_{11}H_2$ and $C_{18}H_{37}N(CH_2CH_2O)_{12}H$, respectively. There are peaks between $C_{18}H_{37}N(CH_2CH_2O)_{x+y}H_2$ and $C_{18}H_{37}N(CH_2CH_2O)_{x+y+1}H_2$ was shown in Figure 4.5.

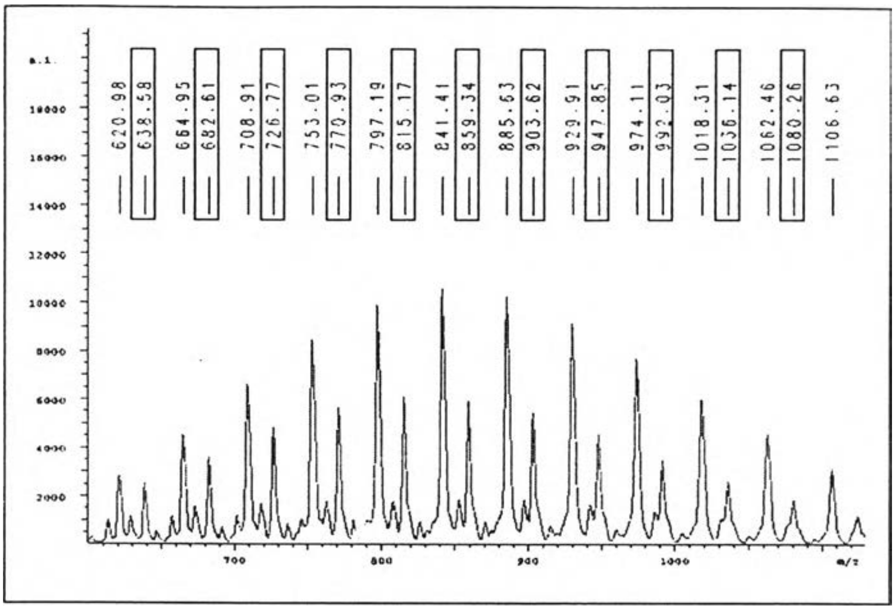


Figure 4.5 MALDI-MS spectrum of alkylamine ethoxylate standard (RP II) shown C_{16}-series.

The interpretation of Figure 4.5 uses the same calculation as example 4.3. The peak at m/z of 638.58 corresponds to $C_{16}H_{33}N(CH_2CH_2O)_9\,H_2$. Consequently, at m/z of 682.61, 726.77, 770.93 and 815.17 correspond to $C_{16}H_{33}N(CH_2CH_2O)_{10}H_2$, $C_{16}H_{33}N(CH_2CH_2O)_{11}H_2$, $C_{16}H_{33}N(CH_2CH_2O)_{12}\,H_2$ and $C_{16}H_{33}N(CH_2CH_2O)_{13}\,H_2$, respectively.

Comercial-A

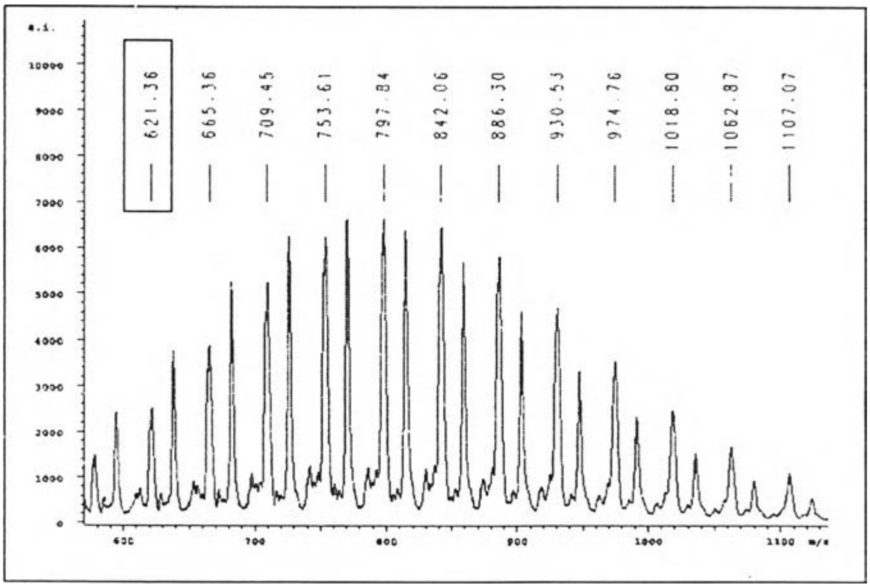


Figure 4.6 MALDI-MS spectrum of alkylamine ethoxylate in commercial-A shown m/z of C_{18}-series.

The interpretation of Figure 4.6 uses the same calculation as example 4.2. The peak at m/z of 621.36 corresponds to $C_{18}H_{37}N(CH_2CH_2O)_8\ H_2$. Consequently, at m/z of 665.36, 709.45, 753.61 and 797.84 correspond to $C_{18}H_{37}N(CH_2CH_2O)_9H_2$, $C_{18}H_{37}N(CH_2CH_2O)_{10}H_2$, $C_{18}H_{37}N(CH_2CH_2O)_{11}\ H_2$ and $C_{18}H_{37}N(CH_2CH_2O)_{12}\ H_2$, respectively. The mass spectrum C16-series was observed like the mass spectrum of alkylamine ethoxylate standards.

Commercial-B

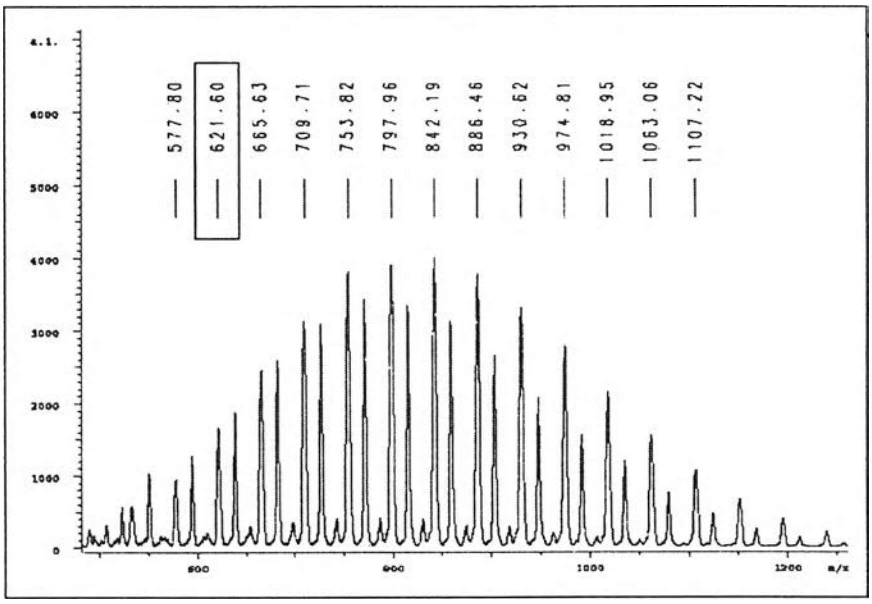


Figure 4.7 MALDI-MS spectrum of alkylamine ethoxylate in commercial-B shown m/z of C_{18}-series.

The interpretation of Figure 4.7 uses the same calculation as example 4.2. The peak at m/z of 621.60 corresponds to $C_{18}H_{37}N(CH_2CH_2O)_8\ H_2$. Consequently, at m/z of 665.63, 709.71, 753.82 and 797.96 correspond to $C_{18}H_{37}N(CH_2CH_2O)_9H_2$, $C_{18}H_{37}N(CH_2CH_2O)_{10}H_2$, $C_{18}H_{37}N(CH_2CH_2O)_{11}\ H_2$ and $C_{18}H_{37}N(CH_2CH_2O)_{12}\ H_2$, respectively. The mass spectrum showed C16-series like the mass spectrum of alkylamine ethoxylate standards.

Commercial-C

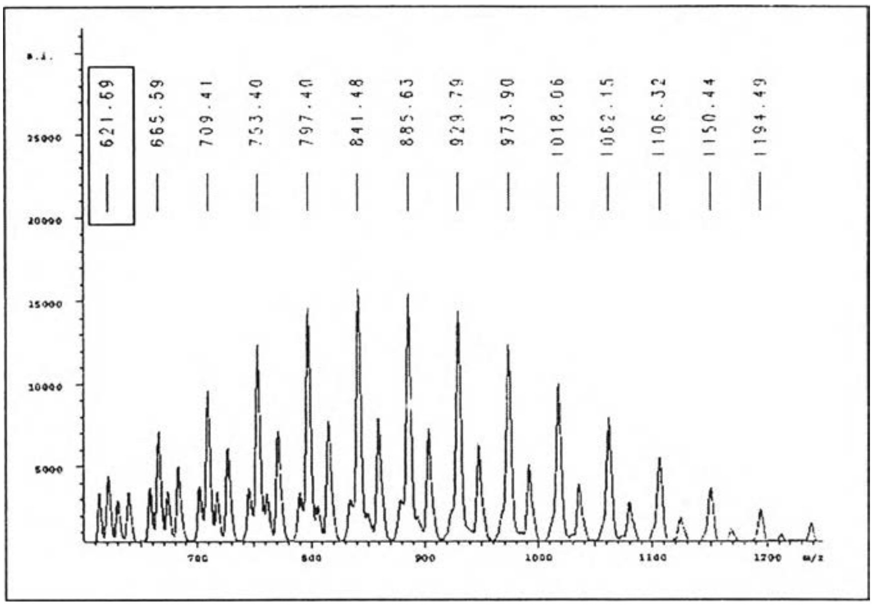


Figure 4.8 MALDI-MS spectrum of alkylamine ethoxylate in commercial-B shown m/z of C_{18}-series.

The interpretation of Figure 4.8 uses the same calculation as example 4.2. The peak at m/z of 621.69 corresponds to $C_{18}H_{37}N(CH_2CH_2O)_8\ H_2$. Consequently, at m/z of 665.59, 709.41, 753.40 and 797.40 correspond to $C_{18}H_{37}N(CH_2CH_2O)_9H_2$, $C_{18}H_{37}N(CH_2CH_2O)_{10}H_2$, $C_{18}H_{37}N(CH_2CH_2O)_{11}\ H_2$ and $C_{18}H_{37}N(CH_2CH_2O)_{12}\ H_2$, respectively. The mass spectrum showed C16-series like the mass spectrum of alkylamine ethoxylate standards.

Commercial-D

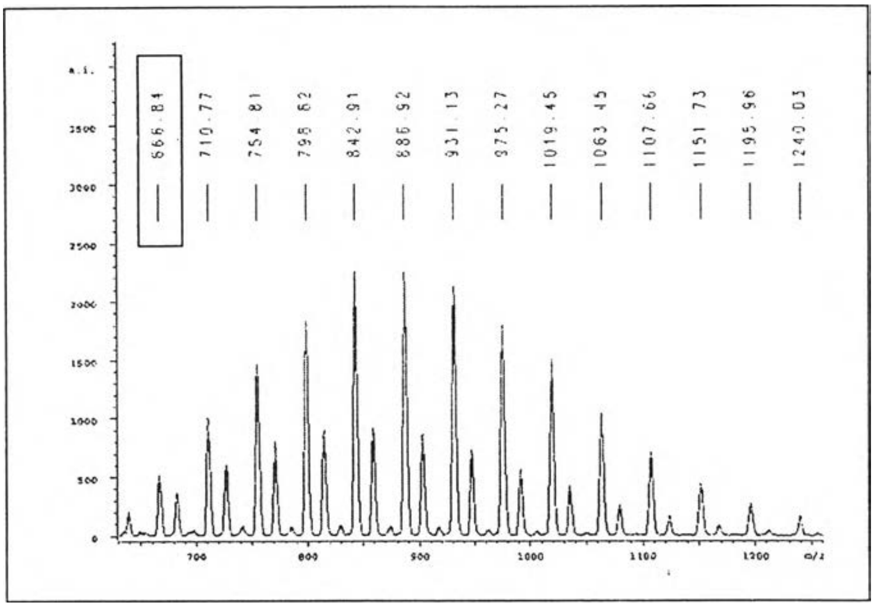


Figure 4.9 MALDI-MS spectrum of alkylamine ethoxylate in commercial-D shown m/z of C_{18}-series.

The interpretation of Figure 4.9 uses the same calculation as example 4.2. The peak at m/z of 666.84.36 corresponds to $C_{18}H_{37}N(CH_2CH_2O)_9\ H_2$. Consequently, at m/z of 710.77, 754.81, 798.82 and 842.91 correspond to $C_{18}H_{37}N(CH_2CH_2O)_{10}H_2$, $C_{18}H_{37}N(CH_2CH_2O)_{11}H_2$, $C_{18}H_{37}N(CH_2CH_2O)_{12}\ H_2$ and $C_{18}H_{37}N(CH_2CH_2O)_{13}\ H_2$, respectively. The mass spectrum showed C16-series like the mass spectrum of alkylamine ethoxylate standards.

Commercial-E

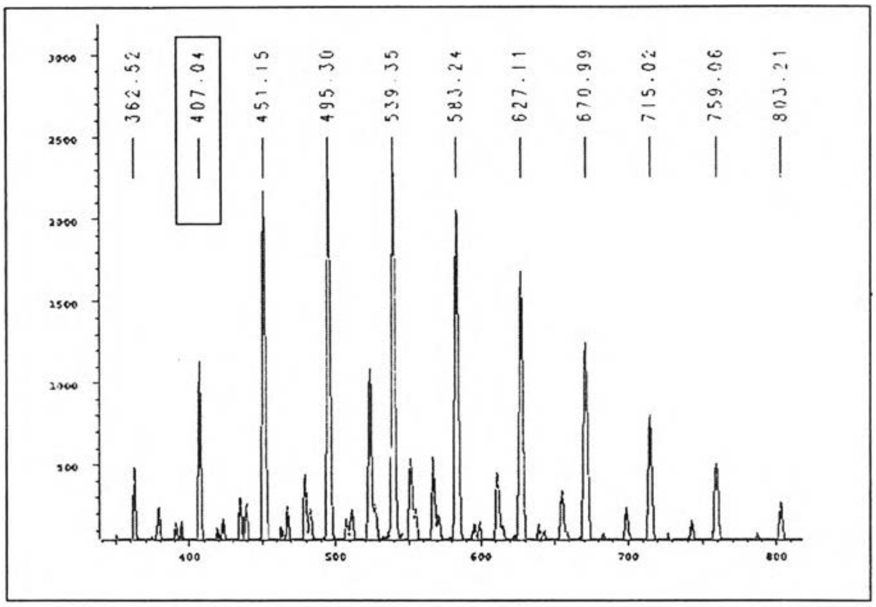


Figure 4.10 MALDI-MS spectrum of alkylamine ethoxylate in commercial-E.

Ex. from eq. 4.3; M + 1 = 407.04

The m/z of 407.04 can not identify to be alkylamine ethoxylate. It corresponds to alcohol ethoxylate; $C_{12}H_{25}(CH_2CH_2O)_5H$. Consequently, at m/z of 451.15, 495.30, 539.35 and 583.24 correspond to $C_{12}H_{25}(CH_2CH_2O)_6H$, $C_{12}H_{25}(CH_2CH_2O)_7H$ and $C_{12}H_{25}(CH_2CH_2O)_8$, respectively.

Detection limit of qualitative and quantitative analysis of alkylamine ethoxylate.

Alkylamine ethoxylate stock solution 10% (TERWET 3780) was diluted in water at concentration ratios 1:20, 1:40, 1:60, 1:80, and 1:100, respectively. All of solutions were mixed CCA matrix with a ratio 1:225.

Calculating 1.0 µg of 1:200 alkylamine ethoxylate and 1:225 CCA,

$0.1 \times 1/20 \times 1/225 = 2.22 \times 10^{-5}$ µg $= 2.22 \times 10^{-11}$ g $= 2.22 \times 10^{-11}/870.22$ femtomole

1.0 µg of 1:200 alkylamine ethoxylate and 1:225 CCA: 25.51 femtomole

1.0 µg of 1:400 alkylamine ethoxyiate and 1:225 CCA: 12.76 femtomole

1.0 μg of 1:600 alkylamine ethoxylate and 1:225 CCA: 8.50 femtomole

1.0 μg of 1:800 alkylamine ethoxylate and 1:225 CCA: 6.44 femtomole

1.0 μg of 1:1000 alkylamine ethoxylate and 1:225 CCA: 5.06 femtomole

The results were shown in Figure A6 (a-e). The figure showed high intensity and polymer distribution clearly which decreased respectively. When, the amount of 5.06 femtomole was loaded (Figure A6 (e)), the signal was low and could not show molecular weight distribution. Thus, Figure A6 (d); 6.44 femtomole was detection limit for qualitative analysis of alkylamine ethoxylate. For limitation of quantitative analysis the amount of alkylamine ethoxylate must more than three times of qualitative analysis which is 19.32 femtomole.

After appropriate conditions for analysis of alkylamine ethoxylate were found and alkylamine ethoxylate standards are same as alkylamine ethoxylate in commercial herbicides. This method can applied for quantitative analysis of alkylamine ethoxylate.

4.3 Quantitative analysis of alkylamine ethoxylate in herbicide samples.

4.3.1 Accuracy from calibration curve which uses neurotensin as an internal standard.

Calibration curve was prepared by diluting stock solution 10% alkylamine ethoxylate (TERWET 3780) in water to concentration of 10.00, 5.00, 2.50, 2.00 and 1.00 percent by and prepare three concentrations were 9.00, 6.50 and 3.00 to compare with concentration which calculate by using equation from calibration curve. All of solutions were mixed with CCA matrix at ratio 1:225. Neurotensin solution was prepared by diluting in water 1 mg/100mL. Finally, neurotensin solution was added to every concentration of alkylamine ethoxylate standard mixtures with amount 1 μL. After loading on target and analysis with MALDI-TOF MS, the spectra were shown in Figure A7 and average intensity of alkylamine ethoxylate and neurotensin were shown in Table 4.5.

Table 4.5 Average intensities of alkylamine ethoxylate and neurotensin from Figure A7-A8.

Concentration of alkylamine ethoxylat (%)	Average intensity of alkylamine ethoxylate (a)	Intensity of neurotensin (n)	a/n
10.00	5173.00	924.00	5.60
5.00	16429.55	5891.00	2.79
2.50	10832.80	9320.00	1.16
2.00	645.20	813.00	0.79
1.00	3782.36	41627.00	0.09
Accuracy test			
9.00	*6931.11*	*1389.00*	*4.99*
6.50	*6620.82*	*1891.00*	*3.50*
3.00	*8991.79*	*6076.00*	*1.48*

Then, concentrations of alkylamine ethoxylate and ratios of average intensity of alkylamine ethoxylate and intensity of interanal standard (neurotensin) were plotted in Figure 4.11.

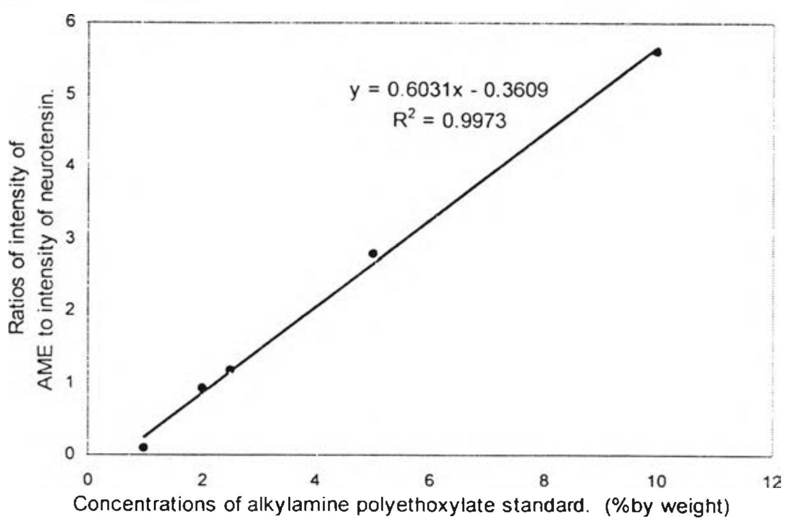


Figure 4.11 Plot of concentrations of alkylamine ethoxylate standard *versus* ratio of intensities of alkylamine ethoxylate to neurotensin from figure 4.5.

From the Figure 4.11, the relationship between concentrations of alkylamine ethoxylate and ratios of average intensity of alkylamine ethoxylate and intensity of internal standard (neurotensin) is linear by R^2= 0.9973 and the equation was y = 0.6031x – 0.3609. The mass spectra for accuracy test were shown in Figure A8 and intensity of alkylamine ethoxylate and internal standard (neurotensin) were shown in Table 4.5. The mass spectrum in Figure A8 (a), given ratio of average intensity of alkylamine ethoxylate and intensity of neurotensin was 4.99 which can calculate be concentration by using equation in figure 4.11.

Example;

$$y = 0.6031x - 0.3609$$

y = ratio of average intensity of alkylamine ethoxylate and intensity of neurotensin.

X = concentration of alkylamine ethoxylate.

When, y = 4.99 then, x = 8.87

Real concentration = 9.00 % and Calculated concentration = 8.87 %, so percent error is

$$[(9.00 - 8.87)/9.00] \times 100 = 1.4 \%$$

According to, y = 3.5 and y = 1.48 from the Figure A9 (b and c), percent error are 1.17 and 1.7, respectively. Then the accuracy test can prove calibration curves which were prepared by this method have efficiency.

4.3.2 Quantitative analysis of alkyamine ethoxylate in herbicide samples by using calibration curve which has neurotensin as internal standard.

Commercial-A

Calibration curve was prepared by diluting stock solution 10% alkylamine ethoxylate (TERWET 3780) in water to concentration of 10.00, 5.00, 2.50, 2.00, and 1.00 percent by weight and prepared four commercial-A samples by dilution with ratios of 1:1, 1:2 , 1:3 and 1:5 to quantitative which calculate by using equation from calibration curve. All of solutions were mixed with CCA matrix at ratio 1:225. Neurotensin solution was prepared by diluting in water 1 mg/100mL. Finally, neurotensin solution was added to all of alkylamine ethoxylate standard mixtures and commercial-A sample mixtures with amount 1 μL. After operating, the spectra of TERWET 3780 and commercial-A were shown in Figure A9-A10. Figure A9 shown the relationship of intensities of various concentrations of alkylamine ethoxylate standard and neurotensin internal standard. Figure A10 shown the relationship of intensities of various concentration of commercial-A samples and neurotensin internal standard. The average intensities of alkylamine ethoxylate and neurotensin were shown in Table 4.6.

Table 4.6 Average intensities of alkylamine ethoxylate and neurotensin from Figure A9-A10.

Concentration of alkylamine ethoxylate (%)	Average intensity of alkylamine ethoxylate (a)	Intensity of neurotensin (n)	a/n
10.00	1924.25	179.00	10.75
5.00	7558.55	1401.00	5.40
2.50	6603.18	2446.00	2.70
2.00	3835.27	1776.00	2.16
1.00	4036.64	4434.00	0.91
Commercial-A 1:1	*6886.18*	*1082.00*	*6.36*
1:2	*3168.91*	*1022.00*	*3.10*
1:3	*724.64*	*359.00*	*2.02*
1:5	*5973.46*	*5015.00*	*1.18*

Then, concentrations of alkylamine ethoxylate and ratios of average intensity of alkylamine ethoxylate and intensity of internal standard (neurotensin) were plotted in Figure 4.12.

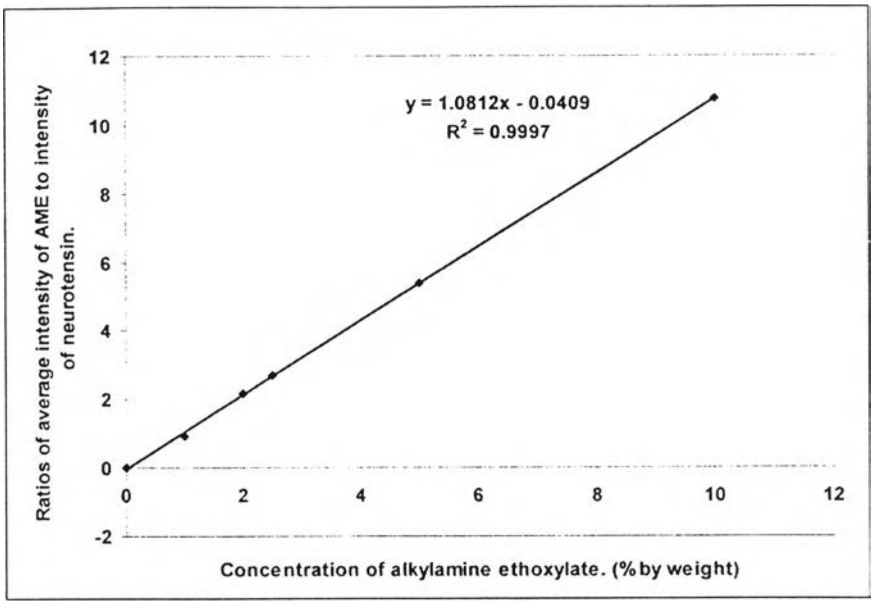


Figure 4.12 Plot of concentrations of alkylamine ethoxylate standard *versus* ratio of intensities of alkylamine ethoxylate to neurotensin from Table 4.6.

From the Figure 4.12, the relationship between concentrations of alkylamine ethoxylate and ratios of average intensity of alkylamine ethoxylate and intensity of neurotensin is linear by R^2= 0.9997 and the equation was y = 1.0842x – 0.0628.

The mass spectra in Figure A10, given ratios of average intensity of alkylamine ethoxylate and intensity of neurotensin were 6.36, 3.10, 2.02 and 1.18 which can calculate be concentration by using equation above.

Example;

$$y = 1.0812x - 0.0409 \quad (4.5)$$

y = ratio of average intensity of alkylamine ethoxylate and intensity of neurotensin.

X = concentration of alkylamine ethoxylate.

When, y = 3.10, 2.02 and 1.18 Then, x = 5.92, 5.81, 5.71 and 5.64

Percent of alkylamine ethoxylate in commercial-A from calculation from the equation of calibration curve was concluded in table 4.6a.

Table 4.6a Concentration of alkyamine ethoxylate in commercial-A before dilution (% by weight)

Ratio of dilution	y	x	Concentration of alkyamine ethoxylate in commercial-A before dilution (% by weight)
1:1	6.36	5.92	5.92
1:2	3.1	2.90	5.81
1:3	2.02	1.90	5.71
1:5	1.18	1.13	5.64

Concentrations of alkylamine ethoxylate in commercial-A which were calculated, are 5.92, 5.81, 5.71 and 5.64 percent by weight. The average concentration of alkylamine ethoxylate is 5.77 percent by weight and standard deviation is 0.12.

Commercial-B

The method for quantitative analysis was prepared same as commercial-A method. Calibration curve was plotted by using alkylamine ethoxylate standard; 10.00, 5.00, 2.5, 2.00 and 1.00 percent by weight and the mass spectra were shown in Figure A11. Commercial-B was diluted at ratio 1:1, 1:2, 1:4 and 1:5 by weight and the mass spectra were shown in Figure A12. The average intensities of alkylamine ethoxylate and neurotensin were shown in Table 4.7.

Table 4.7 Average intensities of alkylamine ethoxylate and neurotensin from Figure A11-A12.

Concentration of alkylamine ethoxylat (%)	Average intensity of alkylamine ethoxylate (a)	Intensity of neurotensin (n)	a/n
10.00	2088.18	158.00	13.22
5.00	5569.00	794.00	7.01
2.50	3168.91	1134.00	2.79
2.00	3912.36	2080.00	1.88
1.00	3260.36	2818.00	1.16

Commercial-B 1:1	*4456.00*	*335.00*	*13.30*
1:2	*2348.80*	*367.00*	*6.40*
1:4	*10168.70*	*3334.00*	*3.05*
1:5	*2967.73*	*1303.00*	*2.28*

Then, concentrations of alkylamine ethoxylate and ratios of average intensity of alkylamine ethoxylate and intensity of internal standard (neurotensin) were plotted in Figure 4.13.

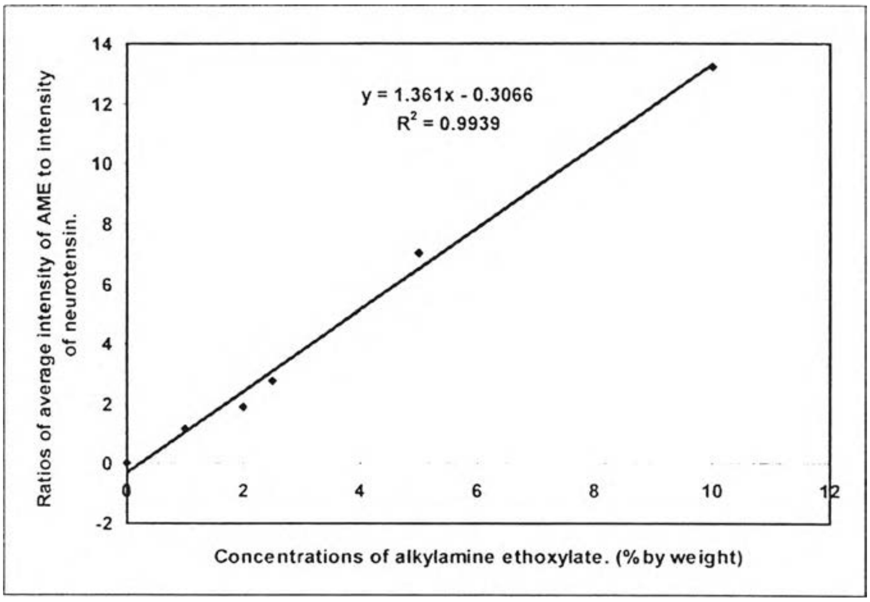


Figure 4.13 Plot of concentrations of alkylamine ethoxylate standard *versus* ratio of intensities of alkylamine ethoxylate to neurotensin from Table 4.7.

From the Figure 4.13, the relationship between concentrations of alkylamine ethoxylate and ratios of average intensity of alkylamine ethoxylate and intensity of neurotensin is linear by R^2= 0.9939 and the equation was y = 1.361x – 0.3066. From this equation and ratios of intensity of alkylamine ethoxylate in commercial-B to intensity of neurotensin, which are 13.30, 6.40, 3.05 and 2.28, were calculated to be concentrations of alkylamine ethoxylate in commercial-B by using the same method as commercial-A. The results were shown in Table 4.7a.

Table 4.7a Concentration of alkyamine ethoxylate in commercial-B before dilution (% by weight)

Ratio of dilution	y	x	Concentration of alkyamine ethoxylate in commercial-B before dilution (% by weight)
1:1	13.30	10.00	10.00
1:2	6.40	4.93	9.86
1:4	3.05	2.47	9.87
1:5	2.28	1.90	9.50

Concentrations of alkylamine ethoxylate in commercial-B which were calculated, are 10.00, 9.86, 9.87 and 9.50 percent by weight. Because the value of 9.50 separated from the others then it was not considered. The average concentration of alkylamine ethoxylate is 9.91 percent by weight and standard deviation is 0.08.

Commercial-C

The method for quantitative analysis was prepared same as commercial-A method. Calibration curve was plotted by using alkylamine ethoxylate standard (RP II); 10.00, 5.00, 2.5, 2.00 and 1.00 percent by weight and the mass spectra were shown in Figure A13. Commercial-C was diluted at ratio 1:2, 1:3, 1:4 and 1:5 by weight and the mass spectra were shown in Figure A14. The average intensities of alkylamine ethoxylate and neurotensin were shown in Table 4.8.

Table 4.8 Average intensities of alkylamine ethoxylate and neurotensin from Figure A13-14.

Concentration of alkylamine ethoxylat (%)	Average intensity of alkylamine ethoxylate (a)	Intensity of neurotensin (n)	a/n
10.00	8991.79	6076.00	1.480
5.00	1817.93	2937.00	0.619

2.50	4498.64	12935.00	0.348
2.00	4918.71	17808.00	0.276
1.00	1348.71	10428.00	0.129
Commercial-C1:2	*6232.93*	*9900*	*0.630*
1:3	*2018.50*	*4450.00*	*0.454*
1:4	*7629.00*	*22400.00*	*0.341*
1:5	*3089.29*	*11700.00*	*0.264*

Then, concentration of alkylamine ethoxylate and ratios of average intensity of alkylamine ethoxylate and intensity of internal standard (neurotensin) was plotted in Figure 4.14.

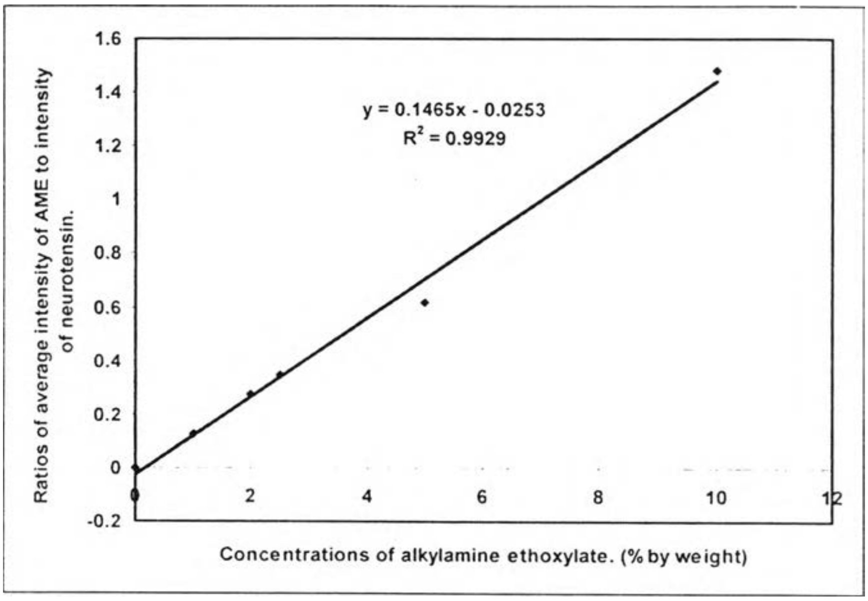


Figure 4.14 Plot of concentrations of alkylamine ethoxylate standard *versus* ratio of intensities of alkylamine ethoxylate to neurotensin from Table 4.8.

From the Figure 4.14, the relationship between concentrations of alkylamine ethoxylate and ratios of average intensity of alkylamine ethoxylate and intensity of neurotensin is linear by R^2= 0.9929 and the equation was y = 0.1465x – 0.0253. From this equation and ratios of intensity of alkylamine ethoxylate in commercial-C to intensity of neurotensin, which are 0.630, 0.454, 0.341 and 0.264, were calculated to be concentrations of alkylamine ethoxylate in commercial-C by using the same method as commercial-A and B. The results were shown in Table 4.8a.

Table 4.8a Concentration of alkyamine ethoxylate in commercial-C before dilution (% by weight)

Ratio of dilution	y	x	Concentration of alkyamine ethoxylate in commercial-C before dilution (% by weight)
1:2	0.630	4.473	8.95
1:3	0.454	3.272	9.82
1:4	0.341	2.500	10.00
1:5	0.264	1.974	9.87

Concentrations of alkylamine ethoxylate in commercial-C which were calculated are 8.95, 9.82, 10.00 and 9.87 percent by weight. Because the value of 8.95 separated from the others then it was not considered. The average and standard deviation are 9.90 and 0.09, respectively.

Commercial-D

The method for quantitative analysis was prepared same as commercial-A method. Calibration curve was plotted by using alkylamine ethoxylate standard (RP II); 5.00, 2.5, 2.00, 1.00 and 0.50 percent by weight and the mass spectra were shown in Figure A15. Commercial-D was diluted at ratio 1:1 and 1:2 by weight and the mass spectra were shown in Figure A16. The average intensities of alkylamine ethoxylate and neurotensin were shown in Table 4.9.

Table 4.9 Average intensities of alkylamine ethoxylate and neurotensin from Figure A15-16.

Concentration of alkylamine ethoxylat (%)	Average intensity of alkylamine ethoxylate (a)	Intensity of neurotensin (n)	a/n
5.00	8853.79	1913.00	4.628
2.50	2960.86	1497.00	1.978
2.00	1793.71	1325.00	1.354

1.00	2653.84	5450.00	0.486
0.50	2170.43	8885.00	0.244
Commercial-D1:1	*1877.17*	*2939.00*	*0.639*
1:1	*775.64*	*673.00*	*1.122*
1:1	*525.00*	*483.00*	*1.087*
1:2	*1228.21*	*3782*	*0.325*

Then, concentration of alkylamine ethoxylate and ratios of average intensity of alkylamine ethoxylate and intensity of internal standard (neurotensin) was plotted in Figure 4.15.

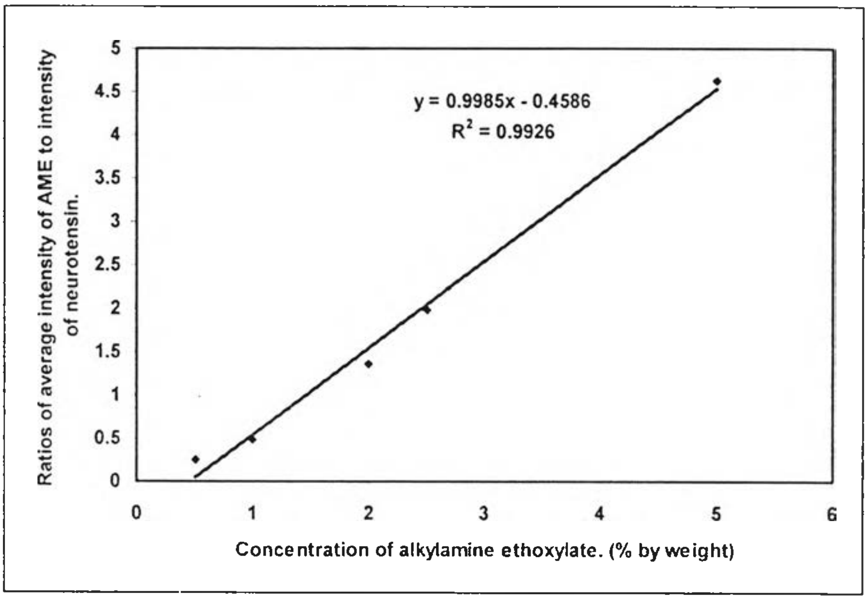


Figure 4.15 Plot of concentrations of alkylamine ethoxylate standard *versus* ratio of intensities of alkylamine ethoxylate to neurotensin from Table 4.9.

From the Figure 4.15, the relationship between concentrations of alkylamine ethoxylate and ratios of average intensity of alkylamine ethoxylate and intensity of neurotensin is linear by R^2= 0.9926 and the equation was y = 0.9985x – 0.4586. From this equation and ratios of intensity of alkylamine ethoxylate in commercial-D to intensity of neurotensin, which are 0.639, 1.122, 1.087 and 0.325, were calculated to be concentrations of alkylamine ethoxylate in commercial-D by using the same method as commercial-A, B and C. The results were shown in Table 4.9a.

Table 4.9a Concentration of alkyamine ethoxylate in commercial-D before dilution (% by weight)

Ratio of dilution	y	x	Concentration of alkyamine ethoxylate in commercial-D before dilution (% by weight)
1:1	0.639	1.102	1.102
1:1	1.122	1.587	1.587
1:1	1.087	1.552	1.552
1:2	0.325	0.787	1.573

Concentrations of alkylamine ethoxylate in commercial-D which were calculated are 1.102, 1.587, 1.552 and 1.573 percent by weight. Because the value of 1.102 separated from the others then it was not considered. The average concentration and standard deviation are 1.571 and 0.017, respectively.