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APPENDICES

APPENDICES: PART I

METHOD VALIDATION

The assay of alkyl-4-hydroxybenzoate and benzodiazepine drugs

I UV-Spectrophotometry

This method was used to determine the aqueous solubility of model substances. They were carried out at the maximum wavelength of 256 and 254 nm for alkyl-4-hydroxybenzoate and benzodiazepine drugs (diazepam and clonazepam), respectively. The calibration curve was constructed by plotting the absorbance against concentrations. The concentration of compounds in aqueous medium was attained from their equations of the calibration curve. The calibration curves of methyl-, ethyl-, propyl- and butylparaben are shown in Appendix: Part I, Figure 1A-4A, respectively. The correlation coefficient values were 0.9998, 0.9999, 1.0000 and 0.9998 for methyl-, ethyl-, propyl- and butylparaben, respectively. In Figures 13A and 14A show the calibration curve of diazepam and lorazepam which gave the correlation coefficient of 0.9998 and 0.9999, respectively. It seemed to be that a very good linear correlation between their concentrations and absorbance was observed.

II High Performance Liquid Chromatography (HPLC)

In this study, HPLC technique was used to determine the oil solubility, partition coefficient, content of compound containing submicron emulsion preparation and amount of compound in the fractional phases. It also used for determining the aqueous solubility of alprazolam and clonazepam. This method was performed to elute four compounds, methyl-, ethyl-, propyl- and butylparaben from the C8 reversed phase column (Symmetry® Waters) at the wavelength of 256 nm. For benzodiazepine drugs analysis, the C18 reversed phase column (Phenomenex®) was performed at the wavelength of 254 nm. A solution of methanol and water in a ratio of 60: 40 was used as a mobile phase for methylparaben and propylparaben analysis while a ratio of 65: 35 was used for ethylparaben and butylparaben analysis. In Figure 5A-8A showed a chromatogram of methyl-, ethyl-, propyl- and butylparaben and its corresponding internal standard, respectively. The retention time of methyl-, ethyl-, propyl- and butylparaben were around 2.38, 2.60, 4.70 and 5.22, respectively. From the data obtained, it was found that the retention time depended on the lipophilicity. The

higher lipophilicity, the longer retention time was observed. So that butylparaben was a higher lipophilic than propyl-, ethyl- and methylparaben. For the analysis of benzodiazepine drugs, a solution of methanol and water in a ratio of 70: 30 was used as a mobile phase. Figures 15A-18A show chromatograms of alprazolam, clonazepam, diazepam and lorazepam with their corresponding internal standards and their retention time values were approximately 5.30, 4.45, 6.50 and 4.80, respectively. This was indicated that the selected mobile phase and also wavelength were appropriate for the analysis of alkyl-4-hydroxybenzoate and benzodiazepine drugs due to a good resolution. The creation of calibration curve, the stock solution of standard solution was diluted with its mobile phase to obtain the final concentrations ranging from 1-10 μg/ml and then these were injected in the volume of 20 μl using auto-sampling apparatus through the HPLC instrument. The peak area ratio of the investigated compounds and the corresponding internal standards were plotted against their concentrations and the correlation coefficient was acquired. The sample concentration was obtained from the equation of the calibration curve.

The HPLC procedure used for quality assessment should meet proper standards of accuracy and reliability. It requires that testing procedure should be validated before used. Typical analytical parameters that should be considered in the validation study were tested as below:

1. System suitability

System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

- Tailing factor

The Tailing factor is specified to measure peak symmetry and its value increases as tailing becomes more pronounced. Tables 1A-4A presented the tailing factor of alkyl-4-hydroxybenzoate compounds while those of benzodiazepine drugs are shown in Tables 18A-21A. From the data obtained, the tailing factor were

less than 2.0 for all compounds. This indicated that peak symmetry was acceptable and hence precision became reliable.

- Resolution

Resolution is defined to measure the closely eluting compound are resolved from each other in order to establish the general resolving power of the system. The requirement of resolution was more than 2.0 (The United States Pharmacopeial Convention 2000). The data of resolution of methyl-, ethyl-, propyl- and butylparaben were 9.40, 10.46, 6.28 and 9.98 respectively while those of alprazolam, clonazepam, diazepam and lorazepam were 3.30, 5.78, 4.23 and 4.25, respectively. Therefore, these operating conditions were suitable for quantitative analysis of alkyl-4-hydroxybenzoate compounds and benzodiazepine drugs since they were clearly resolved from their corresponding internal standards.

- Repeatability

Repeatability expresses the precision under the same operating condition over a short interval of time. It is determined by 6 repeating injections of a homogeneous sample under normal analytical condition and expressed as the % CV and the recommended value should be less than 2.0. Tables 5A and 22A show the repeatability of alkyl-4-hydroxybenzoate and benzodiazepine drugs, respectively. These found that %CV of all compounds were less than 2.0 and indicated that the operating conditions were précised for quantitative analysis of alkyl-4-hydroxybenzoate and benzodiazepine drugs.

2. Accuracy

Since the amount of model compounds/drugs in submicron emulsion or oil phase could not be directly assayed by HPLC method. This due to the interference of oil phase which its retention time was closed to that of the compound of interest e.g. methylparaben and hence reduces the accuracy of the analysis. Therefore, a sample preparation procedure was needed prior to HPLC analysis. Many

studies for extracting such a compound from emulsion including liquid-liquid extractions have been reported (Fitzpatrick, Summa et al. 1975). However, they are time-consuming and expensive. These methods not only require several sample-handling steps but also present the following problems e.g. phase emulsion formation, evaporation of large solvent volumes to concentrate prior to analysis and disposal of toxic and flammable solvents. Recent study, Pongcharoenkiat and co-workers (2003) used solid phase extraction cartridge to separate methylparaben from other components of an oil in water emulsion. This technique was simple, rapid, precise and accurate, required a small volume of solvent and no drying process. Thus, this method was applied in referred work to improve the extraction process providing the higher recovery.

The principle of solid phase extraction (SPE) is similar to that of reversed phase chromatography in which a non-polar stationary phase retains the non-polar compounds including the compound of interesting and allows the polar compounds to pass through. The interference is then removed from the cartridge by the washing solvent which is low eluting power. Finally, the compound of interesting is eluted with a strong eluting power solvent.

In this study, the 3 ml Extrasep™ cartridge was used as the sorbent and methanol was used as the eluting solvent. First, the cartridge must be activated by solving the alkyl chains of sorbent with methanol and then equilibrated with distilled water. The sample was loaded into the cartridge and allowed to pass through the cartridge by gravity flow. In this step, the compound of interesting was retained on the sorbent as well as the interferences. To remove the unwanted compounds, a washing solution, hexane, was added into the cartridge. The eluting power of washing solution is weaker than that of the eluent in order to minimally wash the compound of interesting off the cartridge. Finally, the eluent, methanol, was passed through the cartridge to extract the compound of interesting. The eluate was then diluted with mobile phase and injected into HPLC apparatus. Tables 6A-7A (for alkyl-4-hydroxybenzoate) and Tables 23A-24A (for benzodiazepine drugs) were shown the percentage of extraction recovery of model drugs from oil phase and submicron emulsion base, respectively. The recovery extraction from both systems were more

than 90%, impliedly that the investigated extraction procedure was effective in separatingthe model drugs from other components of an o/w submicron emulsion.

3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the operating conditions. The precision of an analytical method is usually expressed as coefficient of variation in series of measurements. The acceptable value of coefficient of variation was less than 2.0. As shown in Table 9A-12A and Table 26A-29A for alkyl-4-hydroxybenzoate and benzodiazepine drugs, respectively, % CV values of all compounds precision tests were less than 2.0. This revealed that the method used were precise for quantitative analysis in the studied concentration range of alkyl-4-hydroxybenzoate and benzodiazepine drug.

4. Linearity

The linearity is determined by calculating a regression line by the method of least squares of test results versus analyte concentrations. Figure 9A-12A show that the relationship between peak area ratios and concentrations of methyl-, ethyl-, propyl-, and butylparaben were linear with coefficient of determination (R^2) value of 0.9999, 0.9999, 0.9999 and 0.9998, respectively. From Figures 19A-22A, these values of alprazolam, clonazepam, diazepam and lorazepam were 0.9999, 0.9998, 0.9998 and 0.9999, respectively. The results indicated that the HPLC technique was acceptable for quantitative analysis of alkyl-4-hydroxybenzoate and benzodiazepine drug solutions in studied range.

Concentration of methylparaben (μg/ml)	Absorbance
0	0
4	0.413
6	0.626
8	0.826
10	1.045
12	1.264

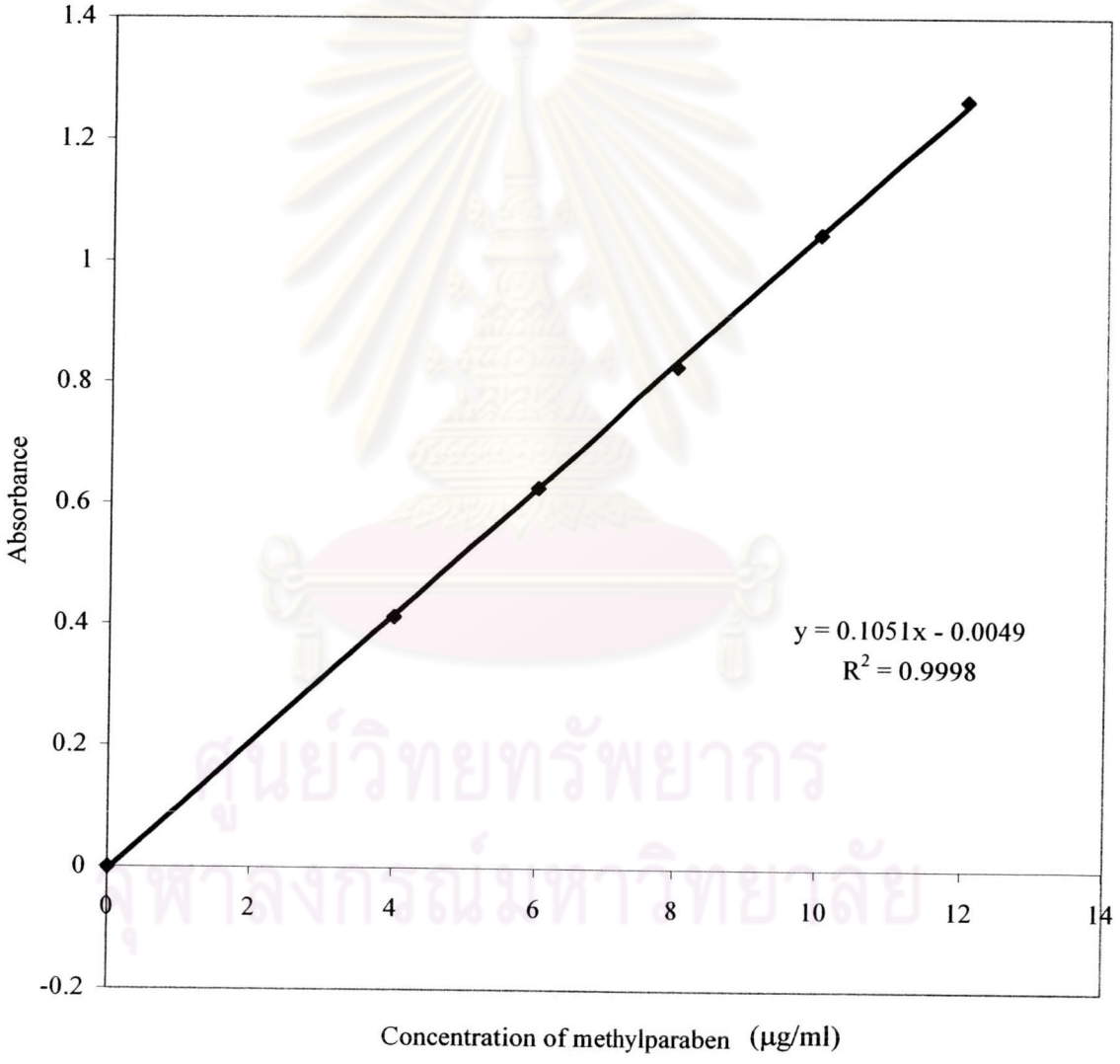


Figure 1A Standard curve of methylparaben

Concentration of ethylparaben (μg/ml)	Absorbance
0	0
2	0.196
4	0.399
6	0.581
8	0.78
10	0.972
12	1.166

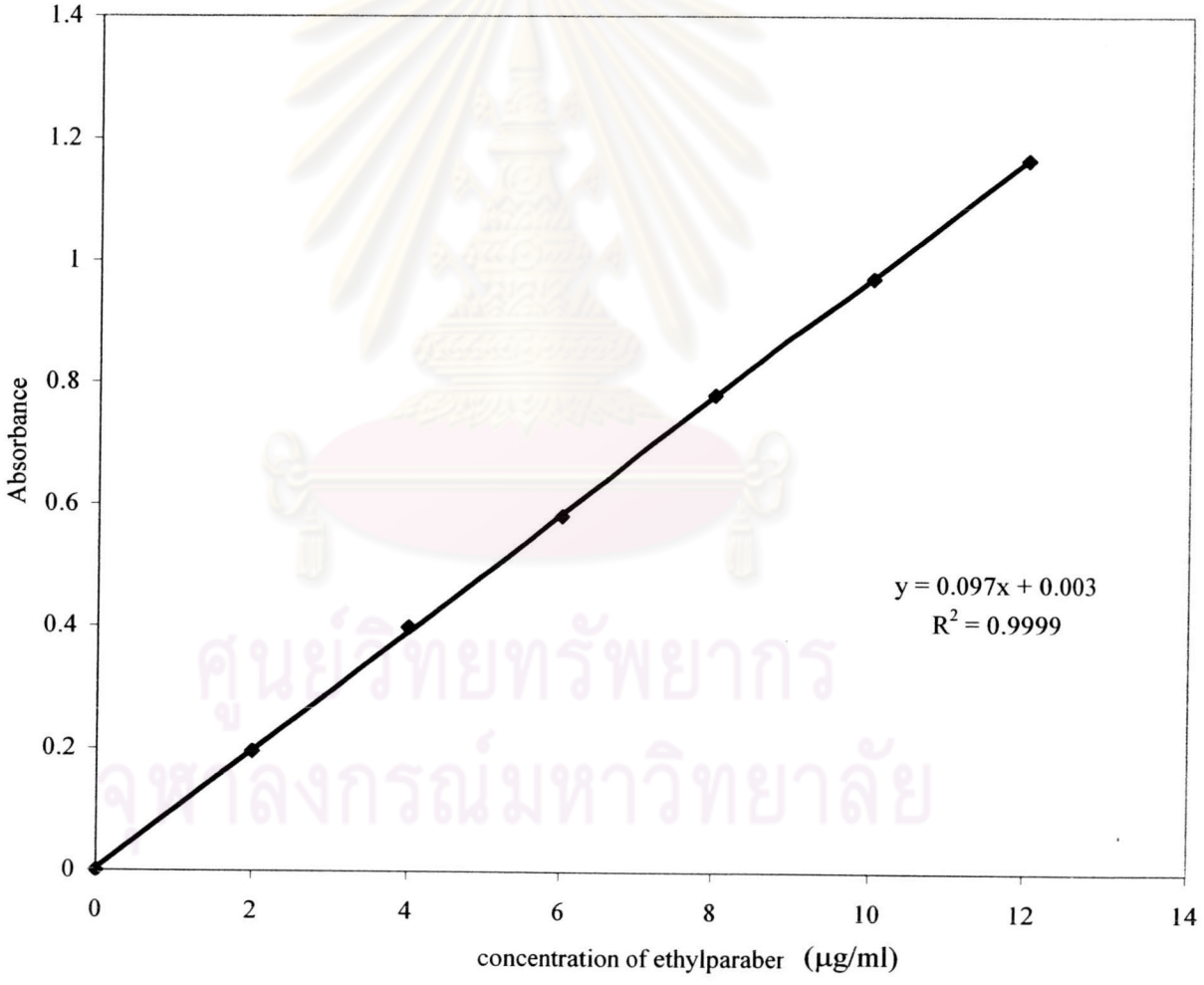


Figure 2A Standard curve of ethylparaben

Concentration of propylparaben (g/ml)	Absorbance
0	0
2	0.187
4	0.36
6	0.539
8	0.719
10	0.896
12	1.07
14	1.247

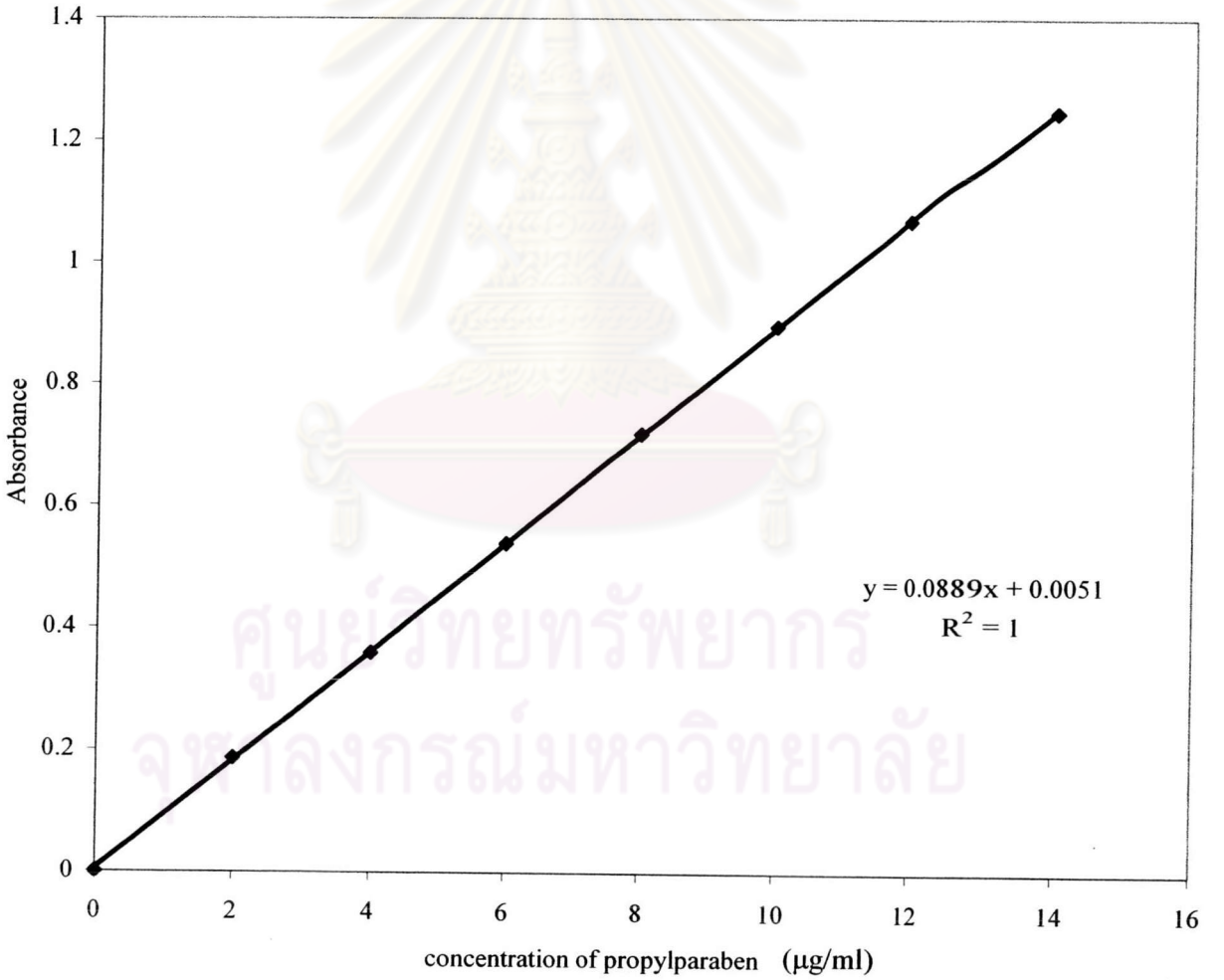


Figure 3A Standard curve of propylparaben

Concentration of butylparaben (μg/ml)	Absorbance
0	0
1	0.08
3	0.24
5	0.401
7	0.567
9	0.716

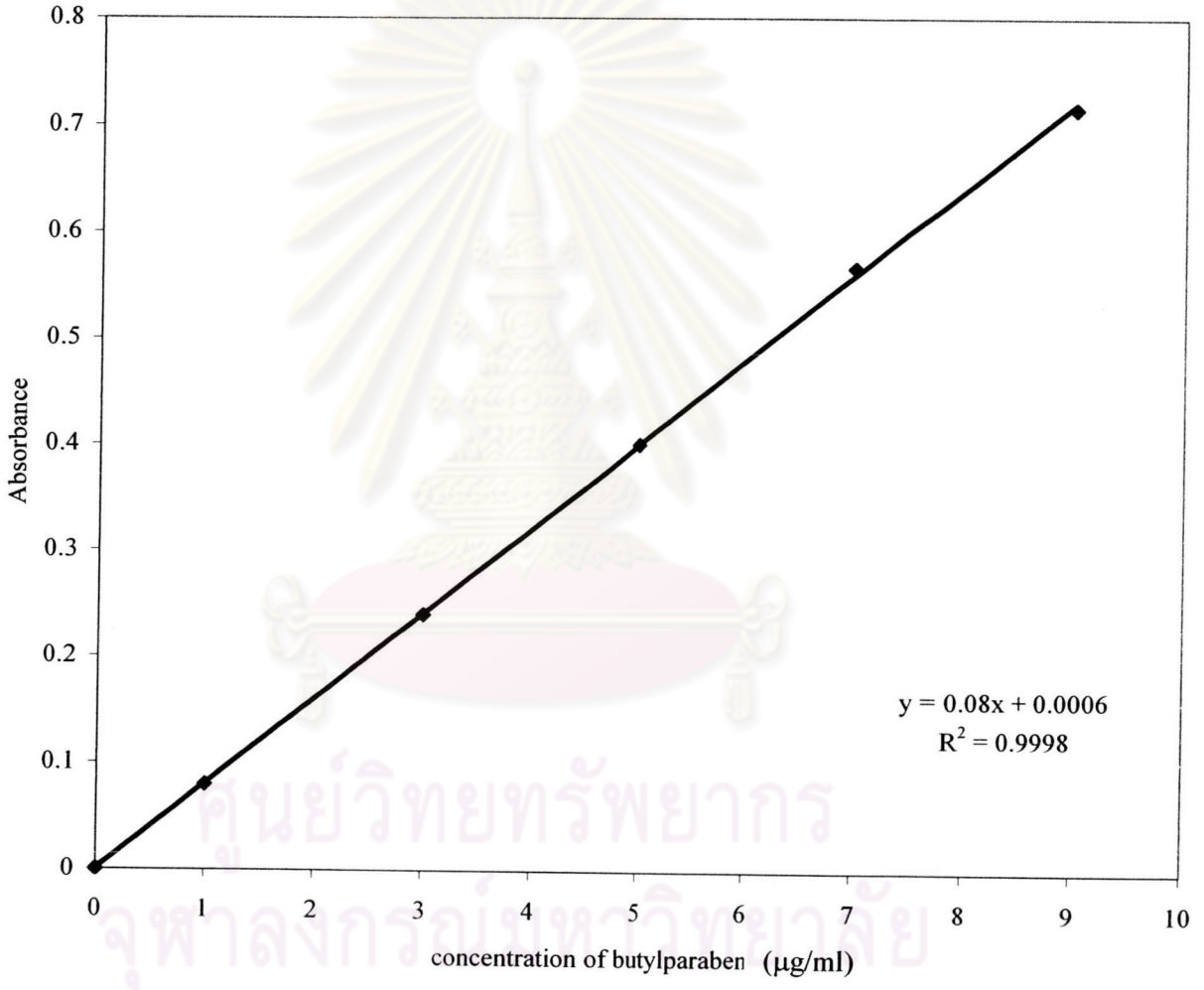


Figure 4A Standard curve of butylparaben

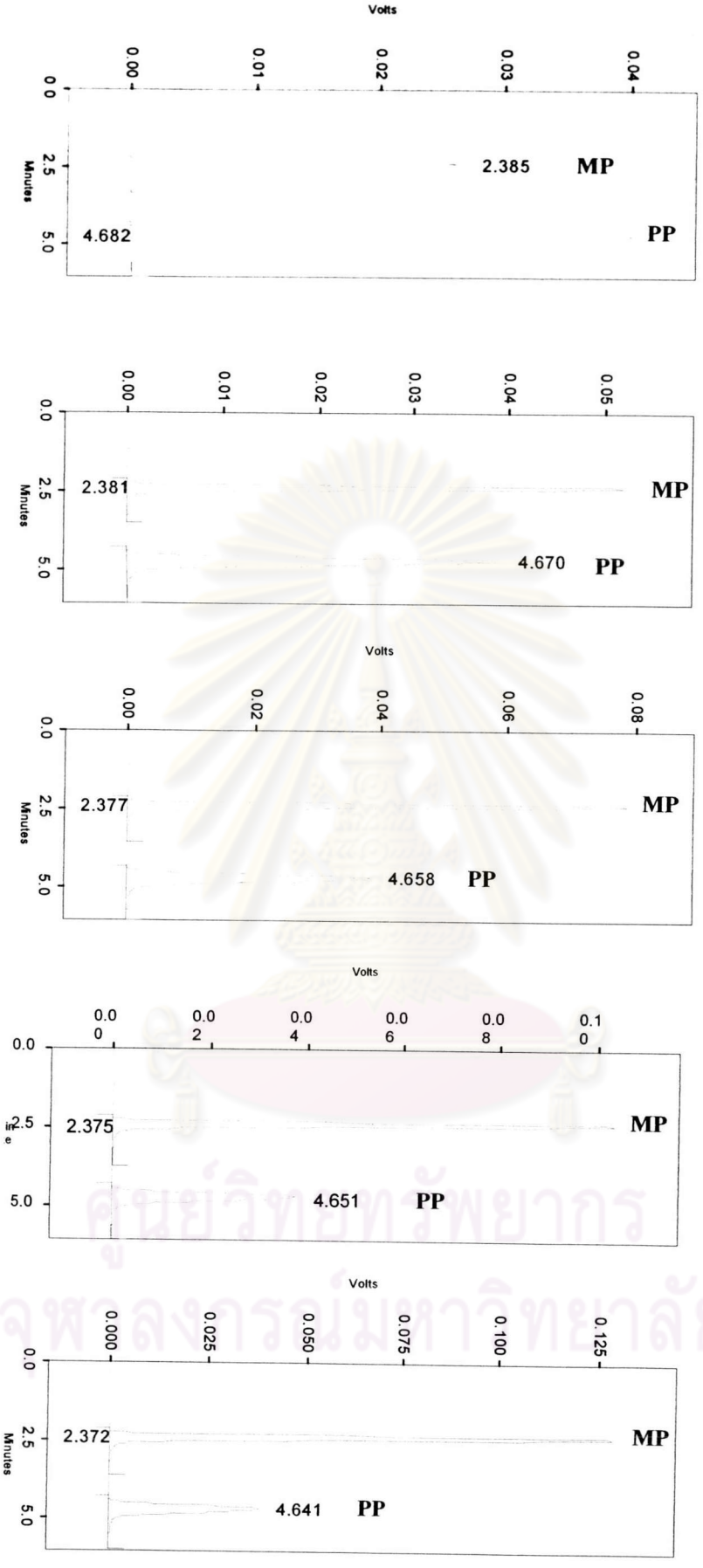
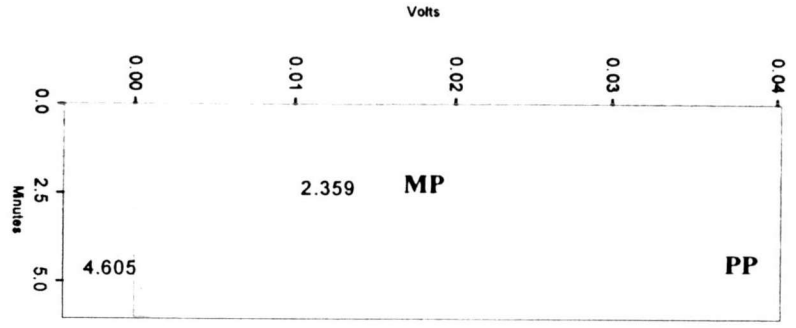
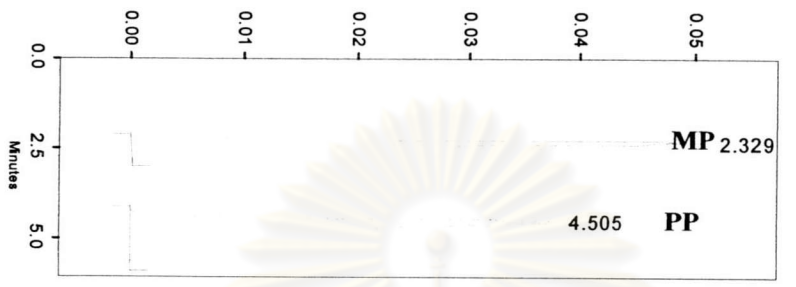


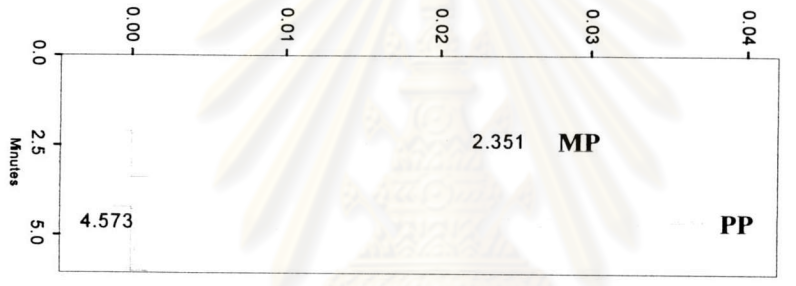
Figure 5A The chromatograms of methylparaben standard solution in various concentration.



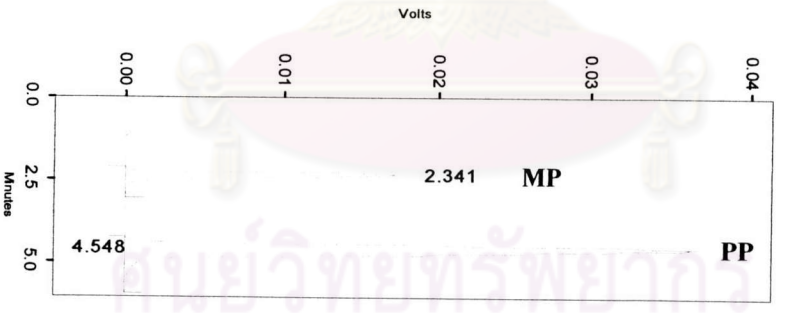
Methylparaben in submicron emulsion



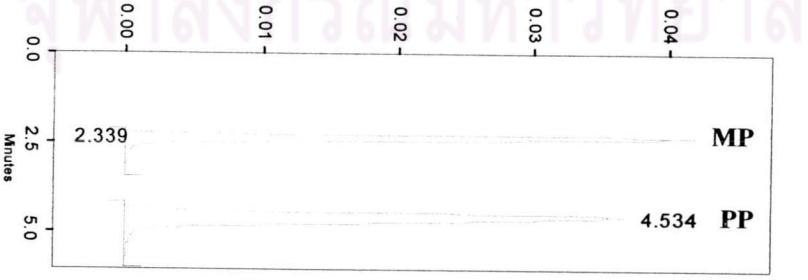
Methylparaben in oil phase



Methylparaben in phospholipid rich phase



Methylparaben in aqueous phase



Methylparaben in mesophase

Figure 5AA The chromatograms of methylparaben in which extracted from the submicron emulsion preparation and different phases.

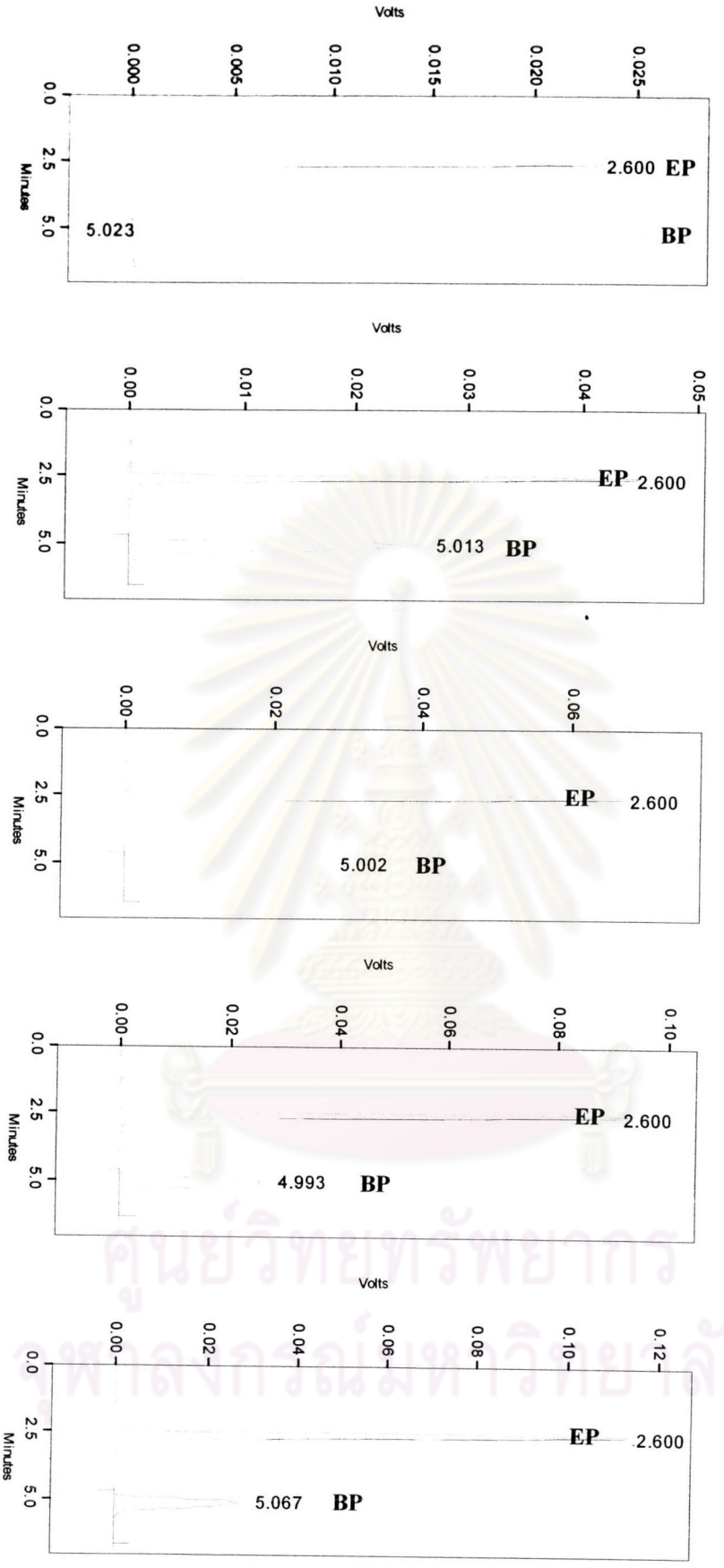


Figure 6A The chromatograms of ethylparaben standard solution in various concentration.

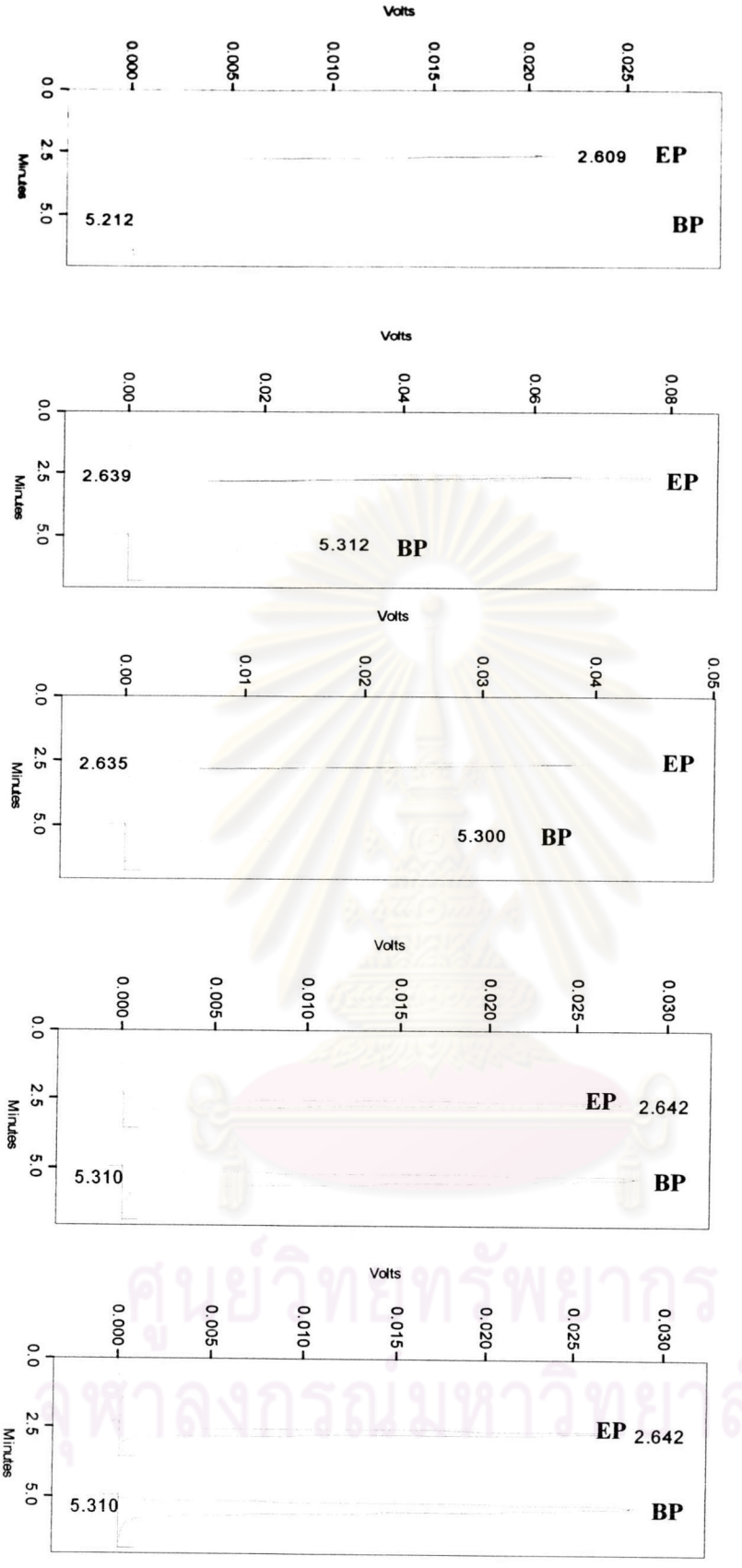


Figure 6AA The chromatograms of ethylparaben in which extracted from the submicron emulsion preparation and different phases.

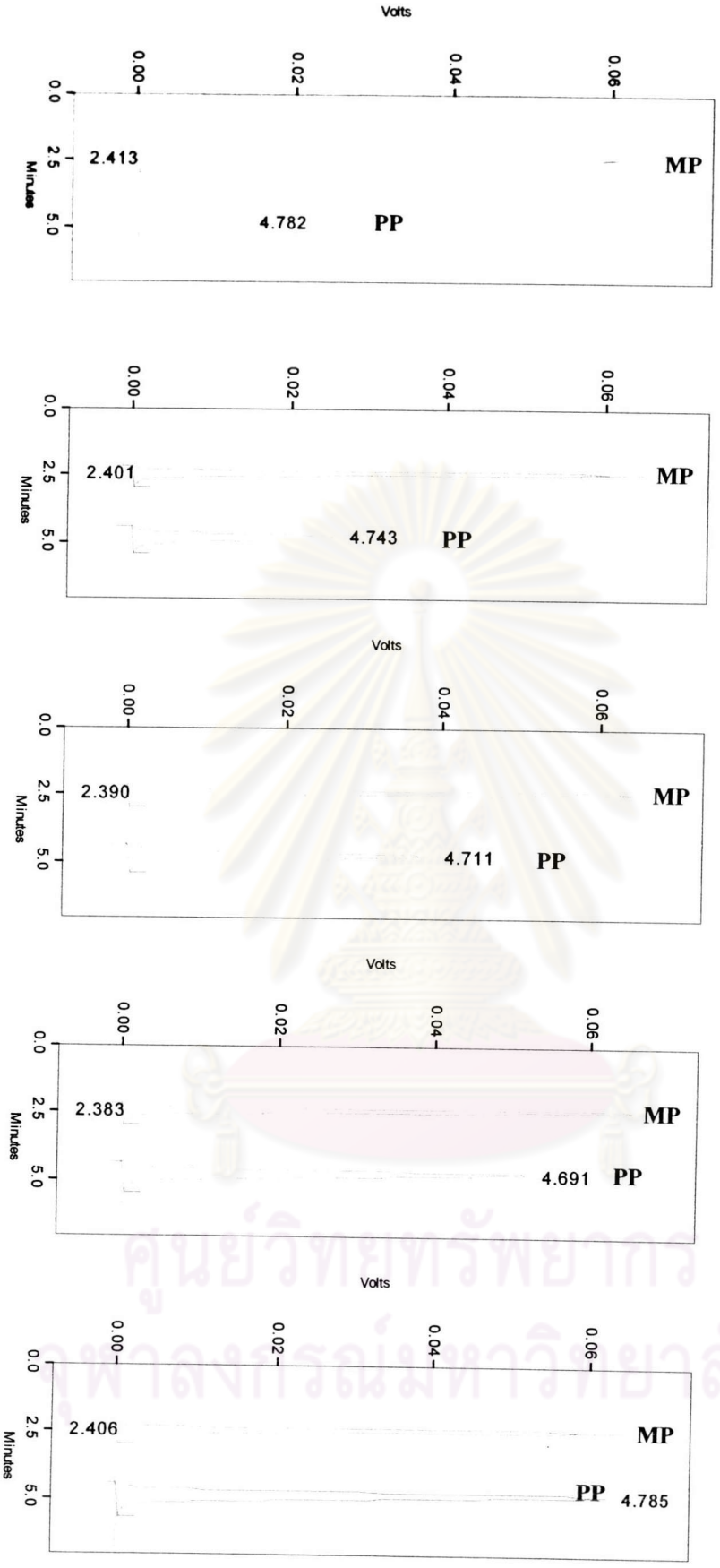
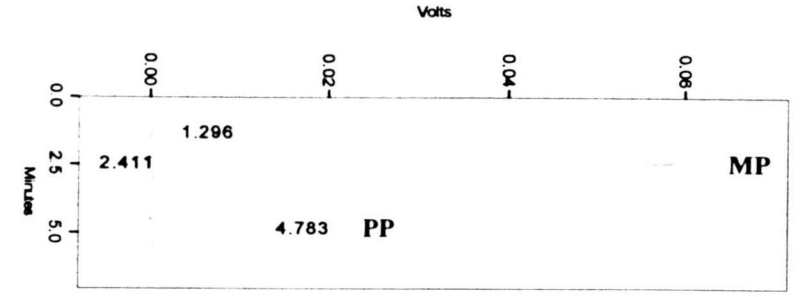
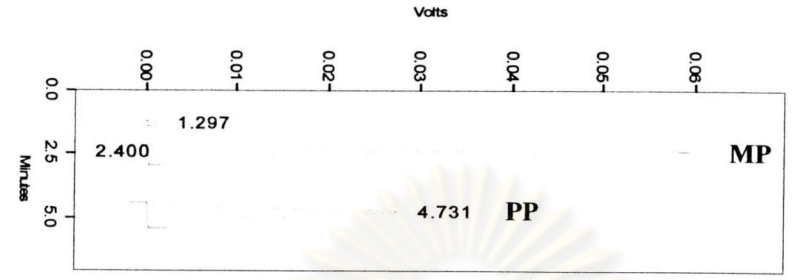


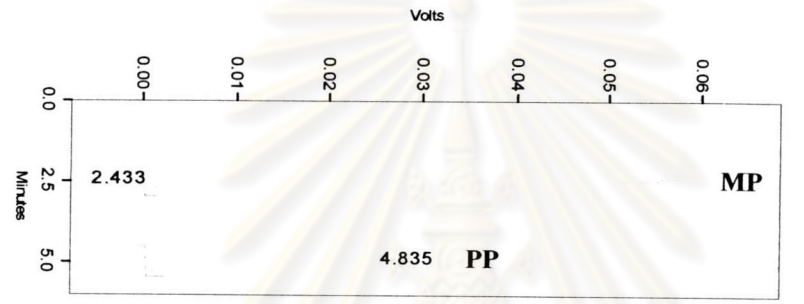
Figure 7A The chromatograms of propylparaben standard solution in various concentration.



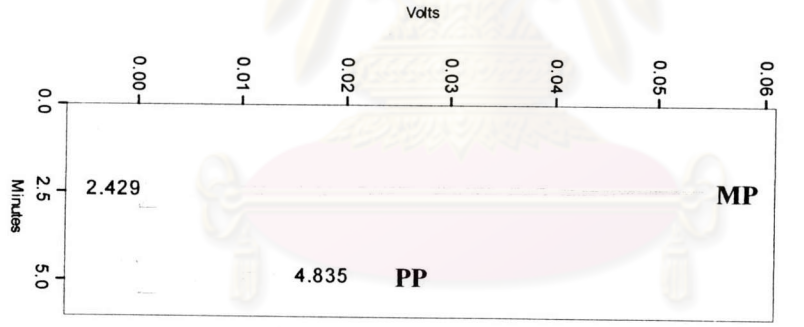
Propylparaben in submicron emulsion



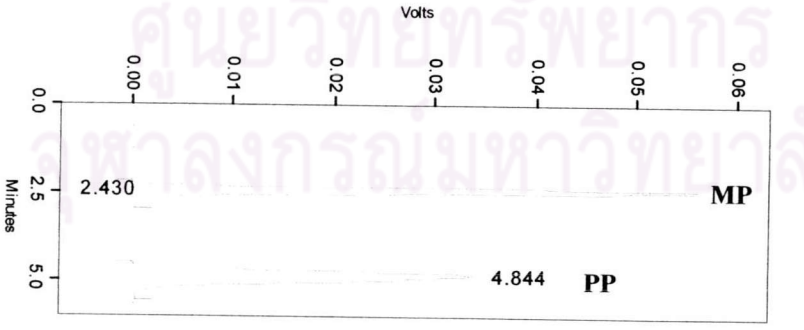
Propylparaben in oil phase



Propylparaben in phospholipid rich phase



Propylparaben in aqueous phase



Propylparaben in mesophase

Figure 7AA The chromatograms of propylparaben in which extracted from the submicron emulsion preparation and different phases.

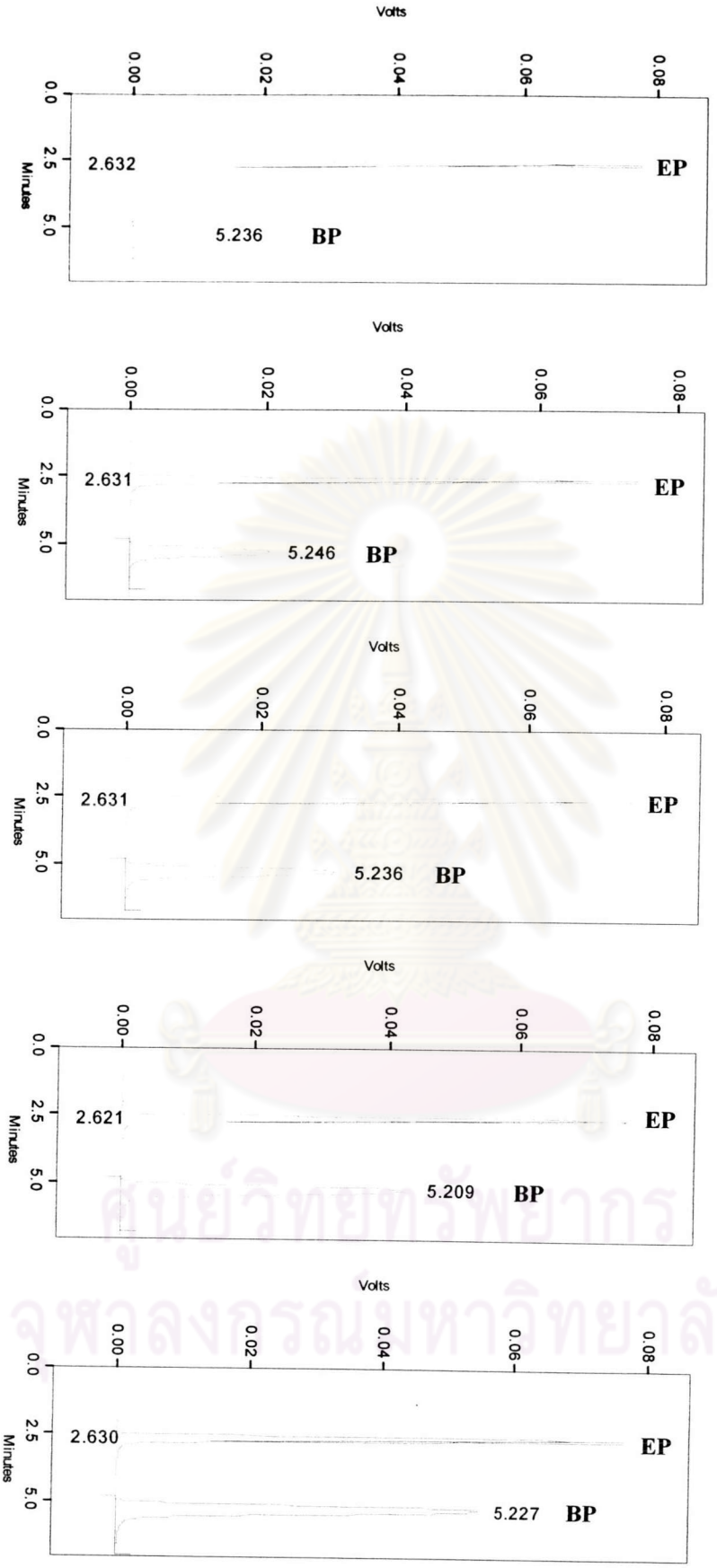
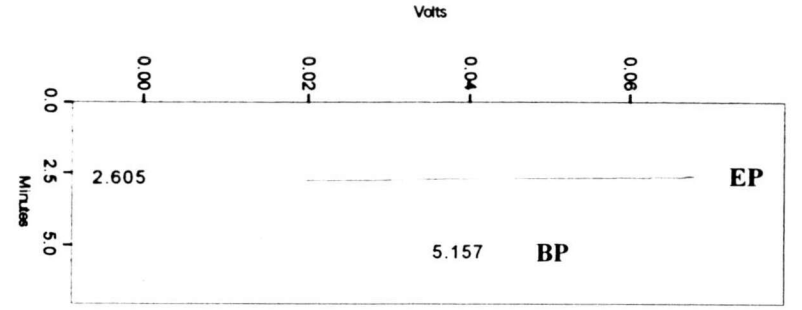
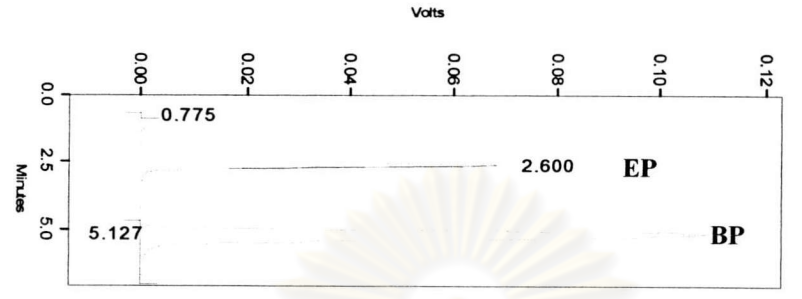


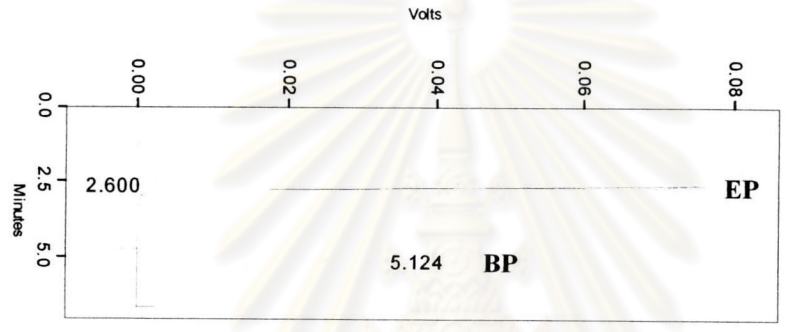
Figure 8A The chromatograms of butylparaben standard solution in various concentration.



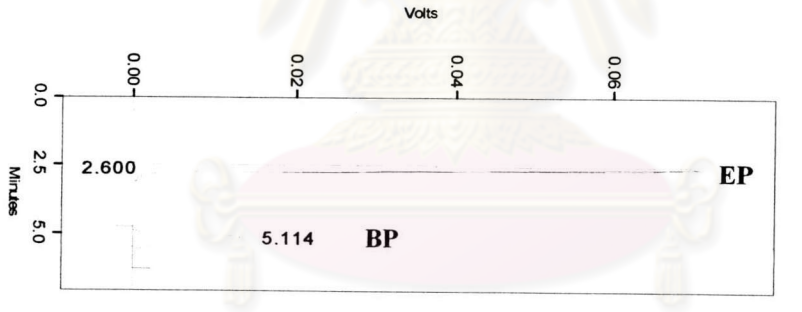
Butylparaben in submicron emulsion



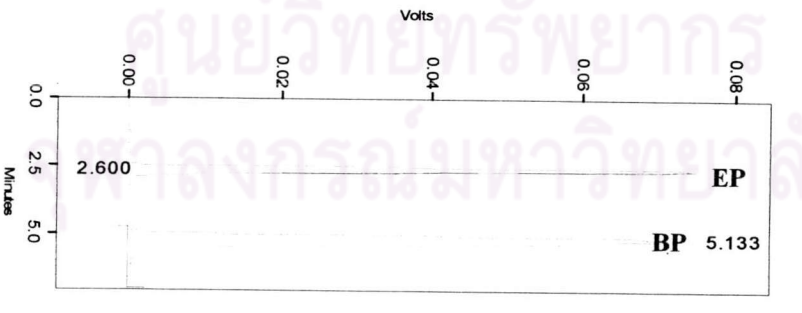
Butylparaben in oil phase



Butylparaben in phospholipid rich phase



Butylparaben in aqueous phase



Butylparaben in mesophase

Figure 8AA The chromatograms of butylparaben in which extracted from the submicron emulsion preparation and different phases.

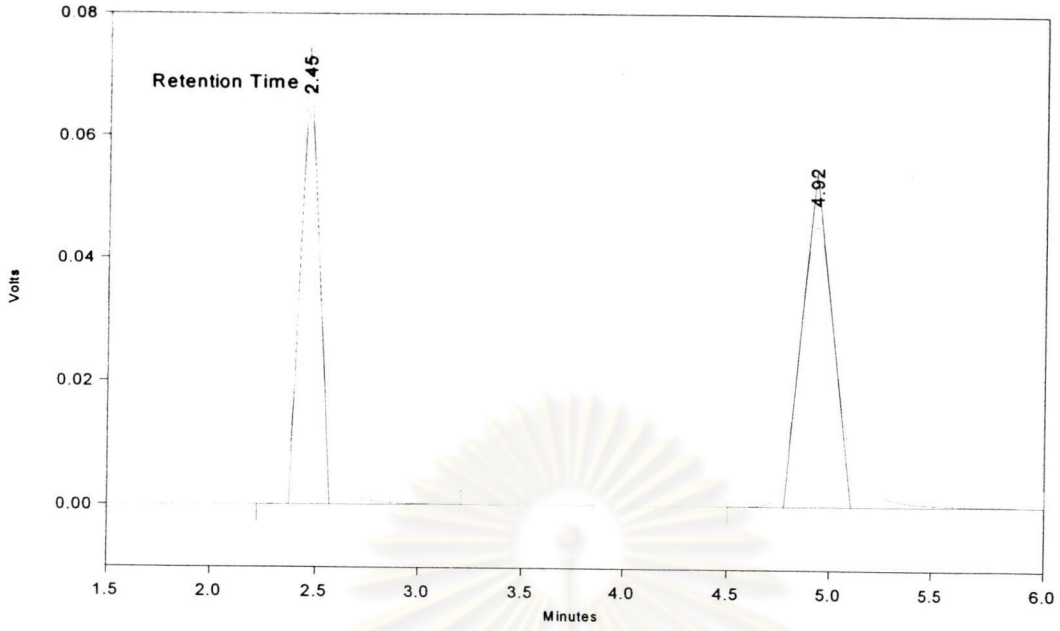


Table 1A Tailing factor and resolution factor of methylparaben and propylparaben.

Detector A (256nm) Name	Retention Time	Area	Resolution	Asymmetry	Asymmetry (10%)
methyl paraben	2.454	486602	0.00	1.41	1.31
propyl paraben	4.923	600181	9.40	1.29	1.20

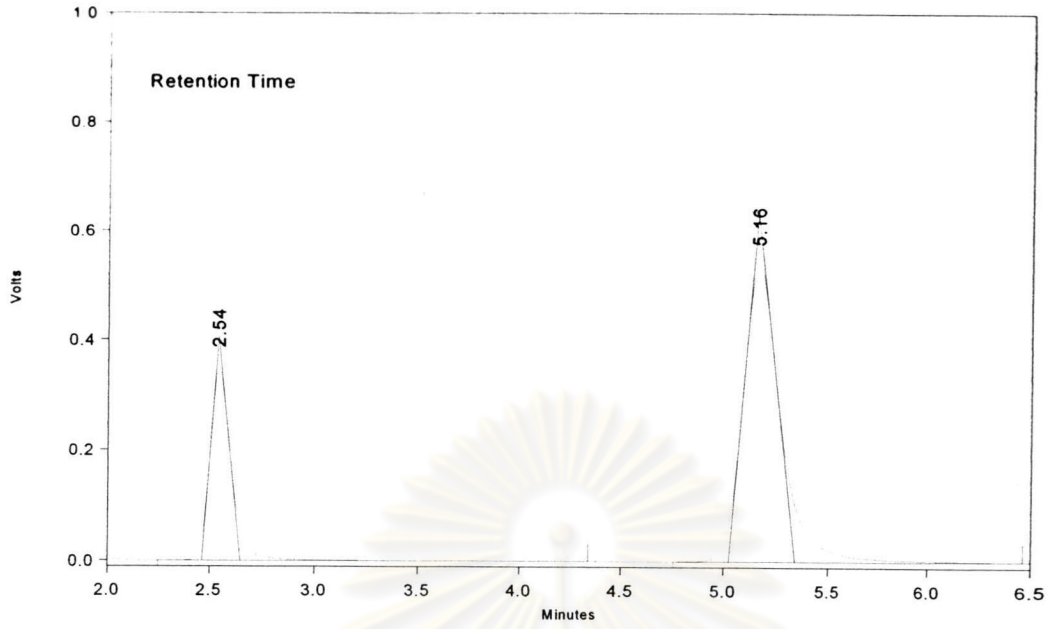


Table 2A Tailing factor and resolution factor of ethylparaben and butylparaben.

Detector A (256nm) Name	Retention Time	Area	Resolution	Asymmetry	Asymmetry (10%)
ethyl paraben	2.538	2555051	0.00	1.43	1.35
butyl paraben	5.159	6801043	10.46	1.41	1.30

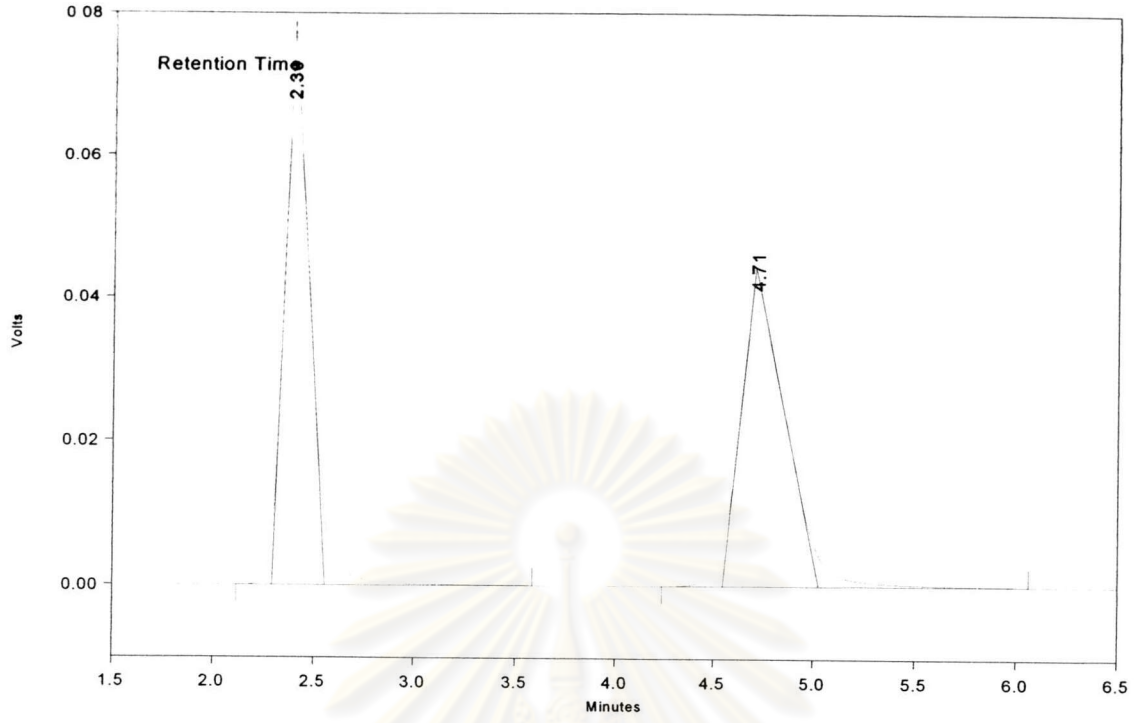


Table 3A Tailing factor and resolution factor of propylparaben and methylparaben.

Detector A (256nm) Name	Retention Time	Area	Resolution	Asymmetry	Asymmetry (10%)
methyl paraben	2.390	659394	0.00	1.41	1.37
propyl paraben	4.711	678512	6.28	1.36	1.36

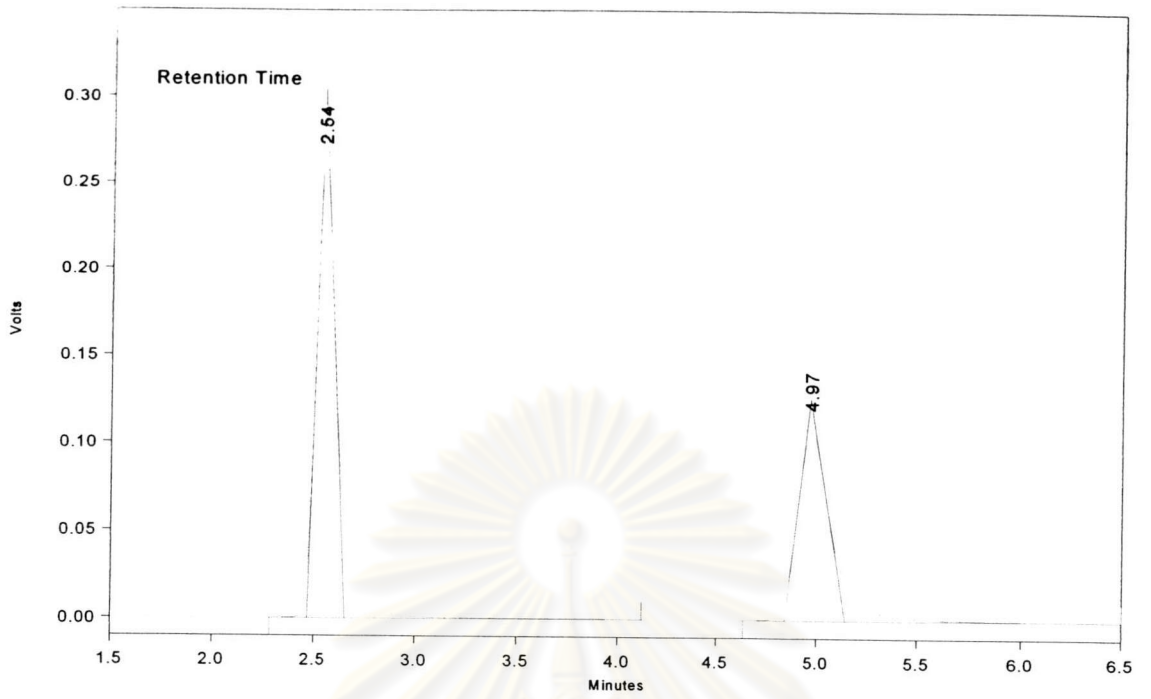


Table 4A Tailing factor and resolution factor of butylparaben and ethylparaben.

Detector A (256nm) Name	Retention Time	Area	Resolution	Asymmetry	Asymmetry (10%)
ethyl paraben	2.541	1957962	0.00	1.62	1.49
butyl paraben	4.966	1319949	9.98	1.54	1.40

Table 5A The repeatability of peak areas of alkyl-4-hydroxybenzoate.

Set No	Peak area ratio			
	Methylparaben	Ethylparaben	Propylparaben	Butylparaben
1	0.9952	0.9510	0.6286	0.6430
2	0.9935	0.9512	0.6292	0.6373
3	0.9944	0.9503	0.6288	0.6398
4	0.9989	0.9565	0.6267	0.6400
5	0.9937	0.9508	0.6301	0.6451
6	0.9953	0.9550	0.6300	0.6389
Average	0.9952	0.9525	0.6289	0.6407
SD	0.0020	0.0026	0.0012	0.0029
%CV	0.1962	0.2736	0.1950	0.4469

Table 6A The recovery of alkyl-4-hydroxybenzoate from soybean oil using solid phase extraction (Extrasep™).

Drug	%Recovery				
	R_1	R_2	Mean	SD	%CV
Methylparaben	101.72	101.53	101.62	0.13	0.13
Ethylparaben	105.28	106.52	105.9	0.88	0.83
Propylparaben	96.06	96.82	96.44	0.54	0.56
Butylparaben	99.40	101.12	100.26	1.22	1.22

Table 7A The recovery of alkyl-4-hydroxybenzoate from lipid emulsion using solid phase extraction (Extrasep™).

Drug	%Recovery				
	R_1	R_2	Mean	SD	%CV
Methylparaben	113.86	113.79	113.82	0.049	0.04
Ethylparaben	107.21	102.68	104.94	3.20	3.05
Propylparaben	108.30	106.51	107.40	1.26	1.17
Butylparaben	103.28	100.99	102.14	1.62	1.59

Table 8A The comparison of %recovery of paraben in various phases between submicron emulsion bases and submicron emulsion containing Arlasolve DMI.

Drug	% Recovery											
	Oil phase			PC rich phase			Aqueous phase			Mesophase		
	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD
Methyl paraben	100.54	93.28	96.91±5.14	101.86	104.04	102.95±1.54	103.51	103.86	103.68±0.24	98.58	99.75	99.17±0.82
	95.65	*99.88*	97.77±2.99	*96.30*	*102.50*	99.40±4.39	*103.68*	*103.00*	103.34±0.48	*101.39*	*101.42*	101.40±0.02
Ethyl paraben	101.11	98.85	99.98±1.60	92.54	97.04	94.79±3.18	101.40	102.15	101.77±0.53	99.48	97.31	98.40±1.53
	97.14	*94.87*	96.00±1.60	*103.42*	*100.46*	101.94±2.09	*99.21*	*99.68*	99.44±0.33	*94.53*	*96.38*	95.45±1.31
Propyl paraben	93.61	90.17	91.89±2.43	108.65	108.26	108.46±0.28	107.34	99.88	103.61±5.28	106.09	104.31	105.20±1.26
	96.02	*94.18*	95.10±1.30	*103.20*	*100.56*	101.88±1.87	*98.48*	*103.24*	100.86±3.37	*103.12*	*103.34*	103.23±0.15
Butyl paraben	90.68	96.26	93.47±3.95	104.66	104.90	104.78±0.17	100.95	107.40	104.18±4.56	99.84	102.34	101.09±1.77
	98.32	*98.41*	98.36±0.06	*100.17*	*100.43*	100.30±0.18	*101.53*	*100.68*	101.11±0.60	*101.94*	*101.53*	101.73±0.29

**% recovery of submicron emulsion containg Arlasolve DMI*

Table 9A The precision of methylparaben (MP).

MP concentration (μg/ml)	Calculated concentration from calibration curve (μg/ml)								
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average	SD	%CV
1.1	1.1094	1.1090	1.1084	1.1059	1.1146	1.1089	1.1094	0.0028	0.2557
4.4	4.3300	4.3230	4.3267	4.3461	4.3239	4.3308	4.3301	0.0084	0.1950
8.8	9.0133	8.9834	8.9933	9.0040	8.9908	8.9880	8.9955	0.0111	0.1235

Table 10A The precision of ethylparaben (EP)

EP concentration (μg/ml)	Calculated concentration from calibration curve (μg/ml)								
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average	SD	%CV
1.08	1.0602	1.0565	1.0580	1.0587	1.0567	1.0594	1.0583	0.0015	0.1390
4.32	4.3338	4.3345	4.3306	4.3585	4.3329	4.3518	4.3404	0.0117	0.2702
8.64	8.7421	8.7224	8.7164	8.6862	8.6856	8.7209	8.7123	0.0222	0.2554

Table 11A The precision of propylparaben (PP)

PP concentration (μg/ml)	Calculated concentration from calibration curve (μg/ml)								
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average	SD	%CV
1.0	0.9556	0.9529	0.9546	0.9538	0.9537	0.9556	0.9544	0.0011	0.1130
4.0	3.9051	3.9083	3.9061	3.8932	3.9144	3.9133	3.9067	0.0076	0.1954
8.0	8.0210	8.0181	8.0263	8.0759	8.0848	8.0118	8.0396	0.0320	0.3979

Table 12A The precision of butylparaben (BP)

BP concentration (μg/ml)	Calculated concentration from calibration curve (μg/ml)								
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average	SD	%CV
1.0	0.9895	0.9922	0.9911	0.9887	0.9950	0.9921	0.9914	0.0022	0.2259
4.0	3.9769	3.9420	3.9571	3.9585	3.9894	3.9518	3.9626	0.0174	0.4389
8.0	7.9971	8.0043	8.0103	8.0294	7.9656	7.9051	7.9853	0.0445	0.5568

Table 13A The linearity of methylparaben

Methylparaben concentration (μg/ml)	Peak area ratio					
	Set No.1	Set No.2	Set No.3	Average	SD	%CV
0	0	0	0	0	0	0
0.55	0.1096	0.1129	0.1141	0.1122	0.0023	2.0621
2.20	0.4406	0.4609	0.4535	0.4517	0.0102	2.2652
4.40	0.9059	0.9231	0.9092	0.9127	0.0091	0.9998
8.80	1.8540	1.8275	1.8239	1.8351	0.0164	0.8945
11.0	2.3182	2.3106	2.3218	2.3169	0.0057	0.2453

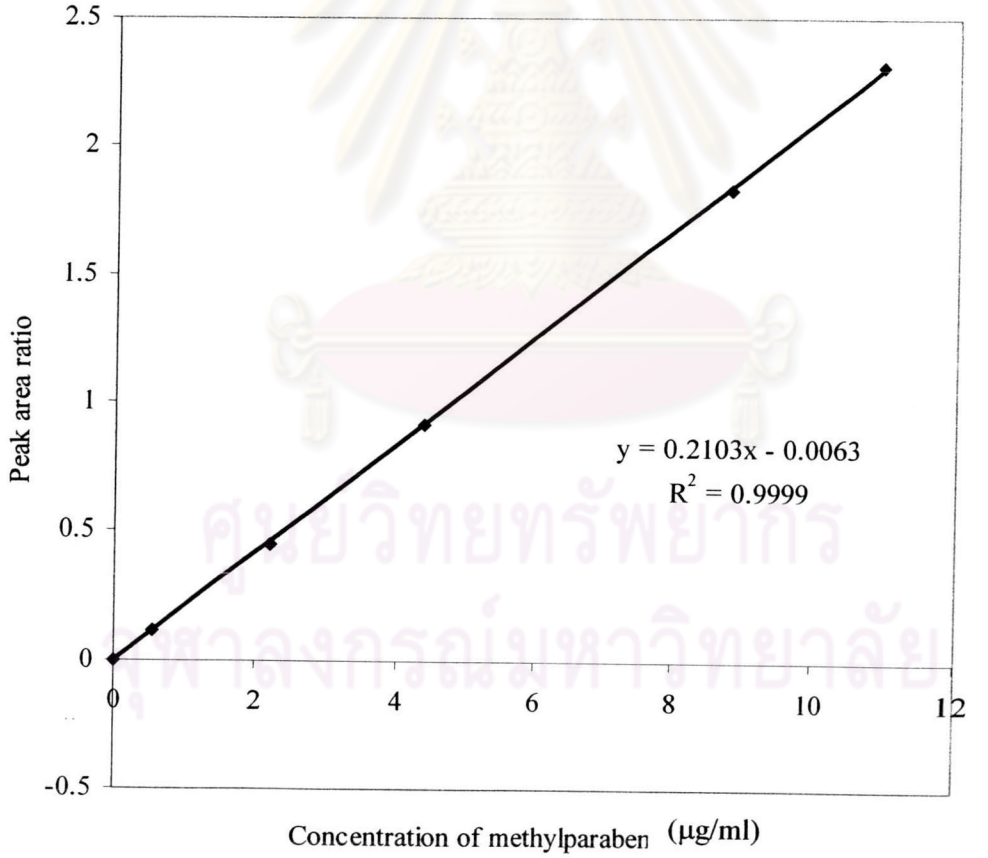


Figure 9A The calibration curve of methylparaben

Table 14A The linearity of ethylparaben

Ethylparaben concentration (μg/ml)	Peak area ratio					
	Set No.1	Set No.2	Set No.3	Average	SD	%CV
0	0	0	0	0	0	0
1.08	0.2302	0.2280	0.2244	0.2275	0.0030	1.2978
2.16	0.4589	0.4549	0.4527	0.4555	0.0032	0.6959
4.32	0.9389	0.9463	0.9478	0.9443	0.0048	0.5040
6.48	1.4371	1.4256	1.4185	1.4270	0.0094	0.6568
8.64	1.9078	1.9254	1.9068	1.9134	0.0105	0.5480

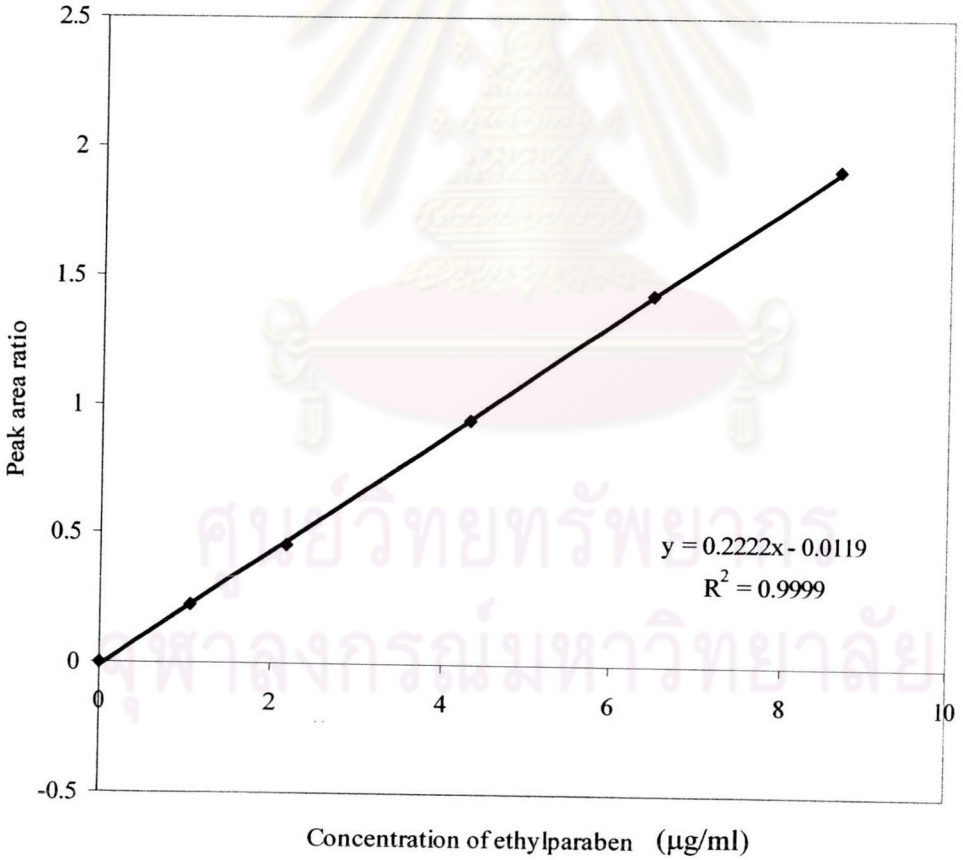


Figure 10A The calibration curve of ethylparaben

Table 15A The linearity of propylparaben

Propylparaben concentration (μg/ml)	Peak area ratio					
	Set No.1	Set No.2	Set No.3	Average	SD	%CV
0	0	0	0	0	0	0
1	0.1560	0.1577	0.1500	0.1546	0.0040	2.5915
2	0.3358	0.3361	0.3231	0.3317	0.0074	2.2475
6	0.9506	0.9763	0.9695	0.9655	0.0133	1.3765
8	1.2846	1.2851	1.2944	1.2880	0.0055	0.4301
10	1.6018	1.6015	1.6137	1.6057	0.0069	0.4324

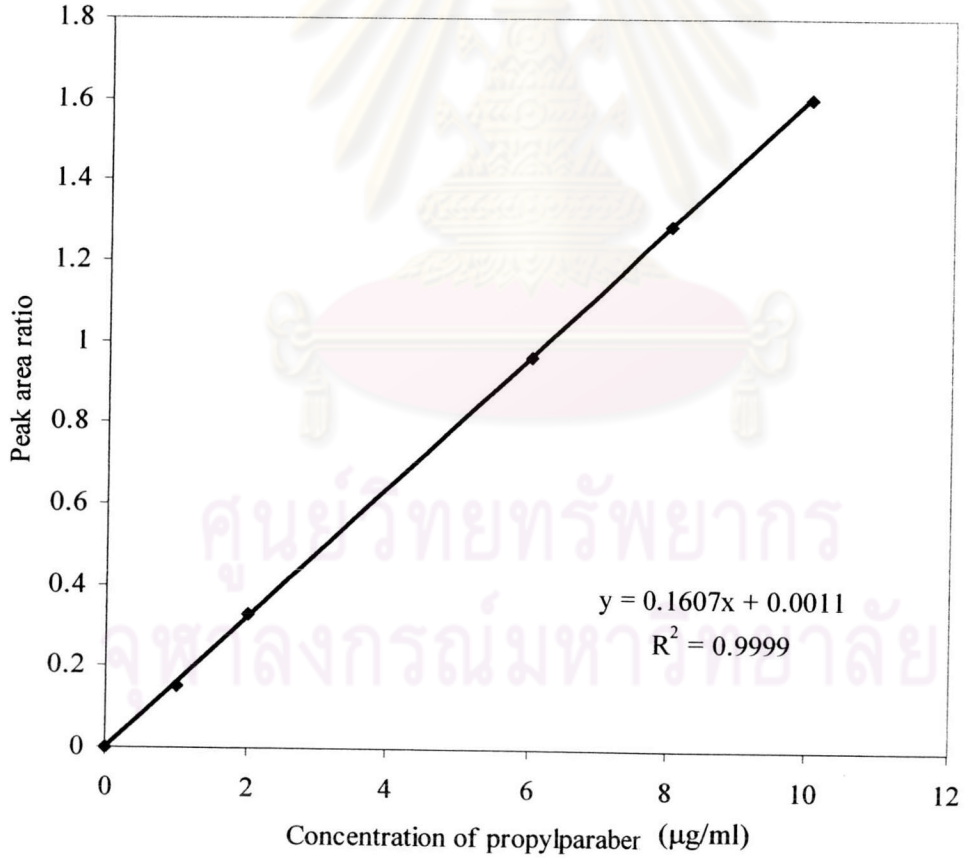


Figure 11A The calibration curve of propylparaben

Table 16A The linearity of butylparaben

Butylparaben concentration (μg/ml)	Peak area ratio					
	Set No.1	Set No.2	Set No.3	Average	SD	%CV
0	0	0	0	0	0	0
1.0	0.1559	0.1512	0.1510	0.1527	0.0028	1.8137
2.0	0.3229	0.3135	0.3201	0.3188	0.0048	1.5110
6.0	0.9616	0.9602	0.9621	0.9613	0.0010	0.1004
8.0	1.3154	1.2987	1.3069	1.3070	0.0084	0.6398
10.0	1.6444	1.6383	1.6583	1.6470	0.0102	0.6216

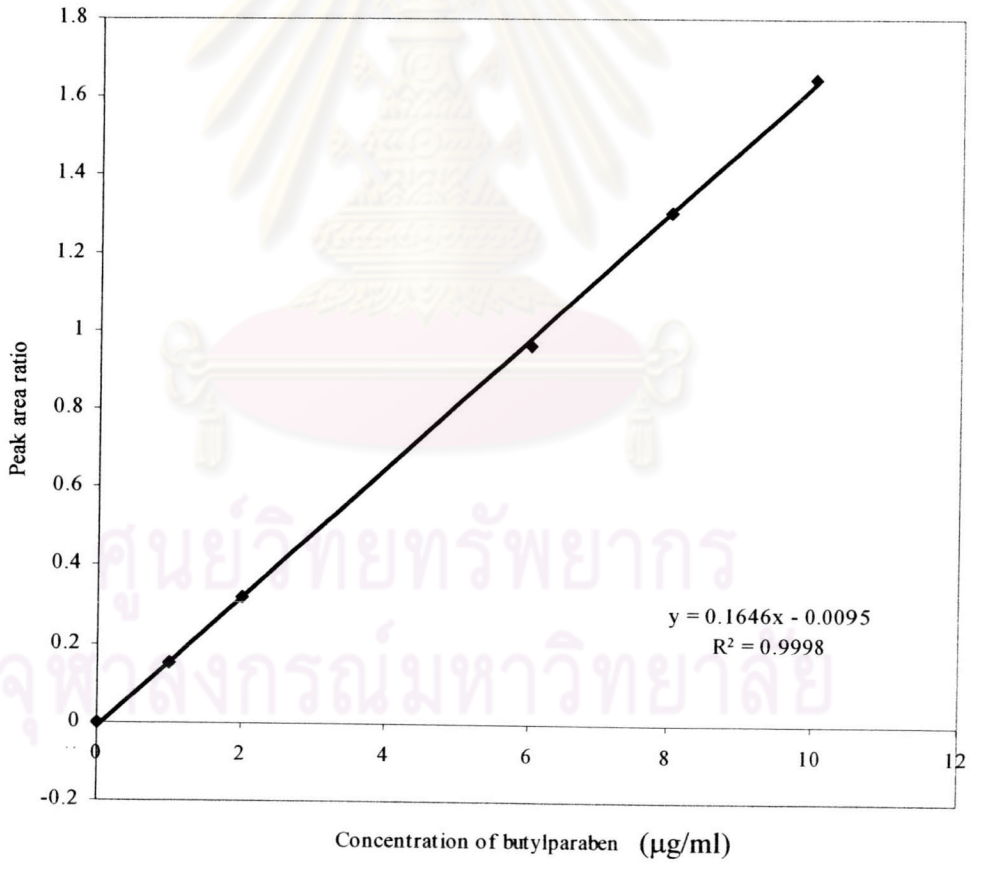


Figure 12A The calibration curve of butylparaben

Table 17A The analytical method validation parameter of HPLC for alkyl-4-hydroxybenzoate.

Parameter	Result value				Limited of acceptability
	Methyl paraben	Ethyl paraben	Propyl paraben	Butyl paraben	
1. System suitability					
- Tailing factor	1.31	1.35	1.36	1.40	≤2
- Resolution factor	9.40	10.46	6.28	9.98	≥2
- Repeatability of peak area (%CV)	0.20	0.27	0.20	0.45	≤2 [a]
2. Specificity	No other peak interfere	No other peak interfere	No other peak interfere	No other peak interfere	No other peak interfere major peak [a]
3. Accuracy %recovery (SD)	101.62 (0.13)	105.9 (0.88)	96.44 (0.54)	100.26 (1.22)	80-110% [b]
4. Precision (%CV)	0.12-0.26	0.14-0.27	0.11-0.40	0.22-0.56	≤ 2 [b]
5. Linearity -The correlation coefficient (r^2)	0.99994	0.99988	0.99993	0.99971	>0.999 [b]

[a] (The United States Pharmacopeial Convention 2000)

[b] (Jenke 1996)

Concentration of diazepam (μg/ml)	Absorbance
0	0
1.76	0.226
3.52	0.446
5.28	0.663
7.04	0.877
8.8	1.083

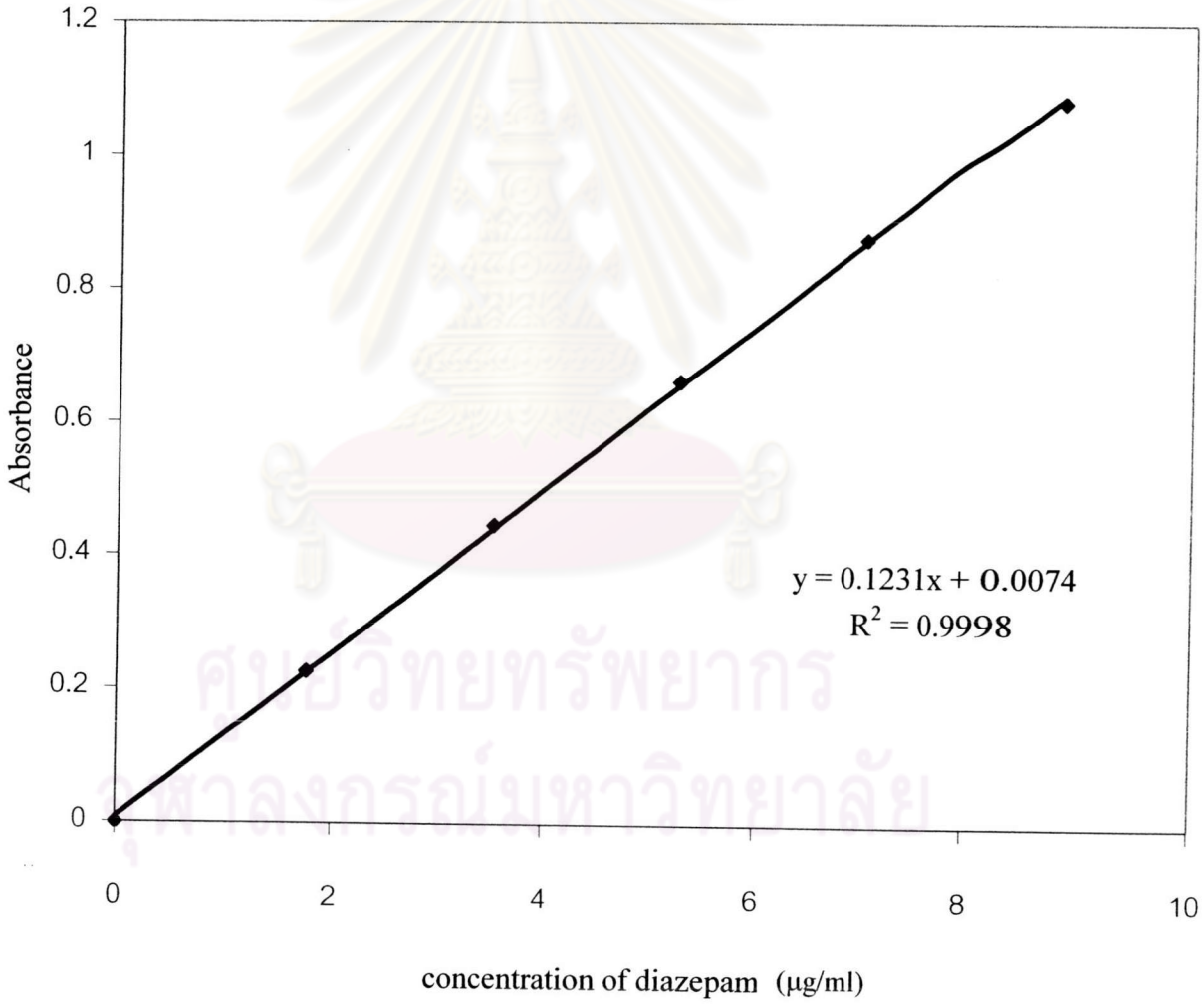


Figure 13A Standard curve of diazepam.

Concentration of lorazepam (μg/ml)	Absorbance
0	0
2.512	0.308
5.024	0.595
7.536	0.878
10.048	1.178
12.56	1.47

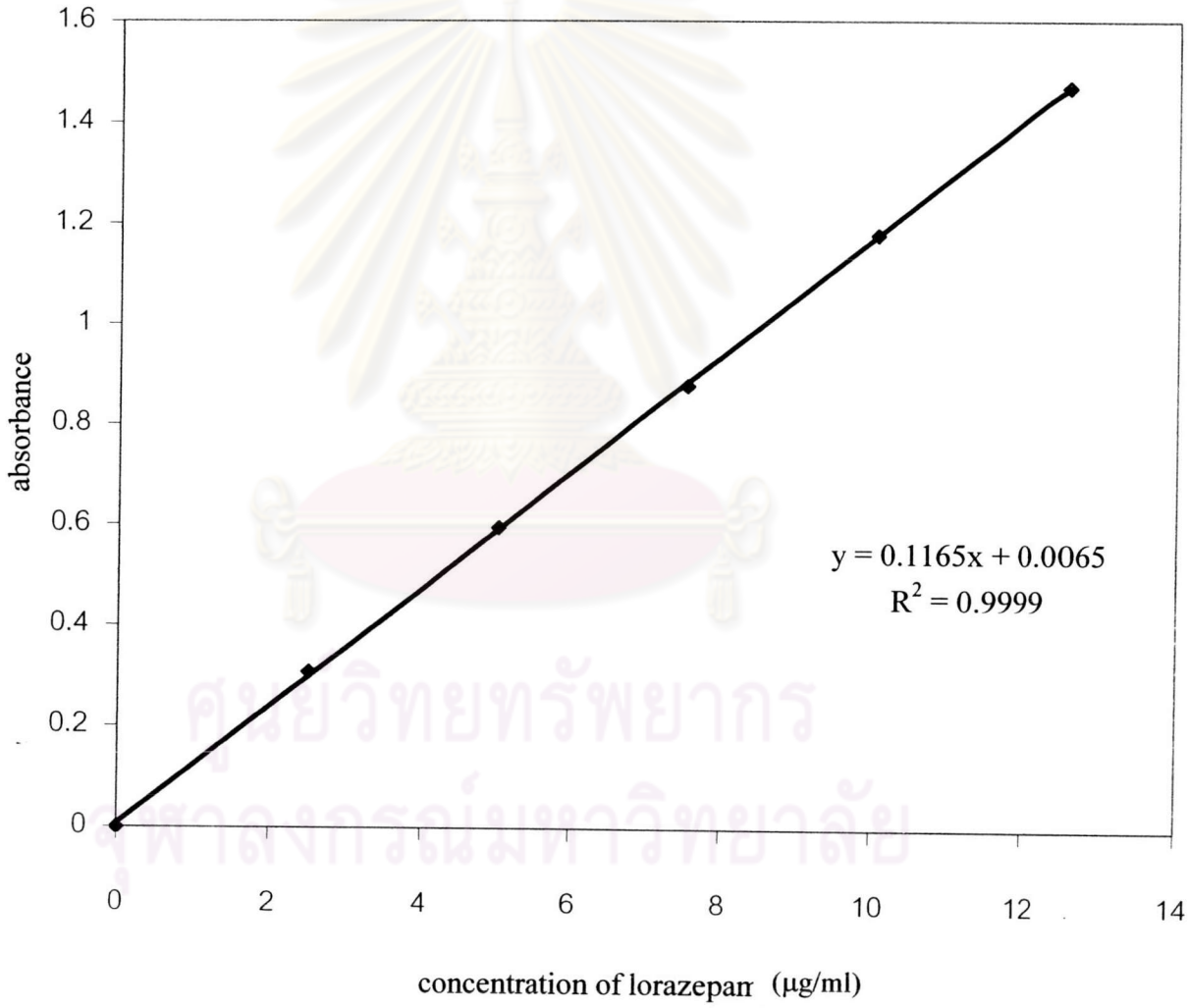


Figure 14A Standard curve of lorazepam.

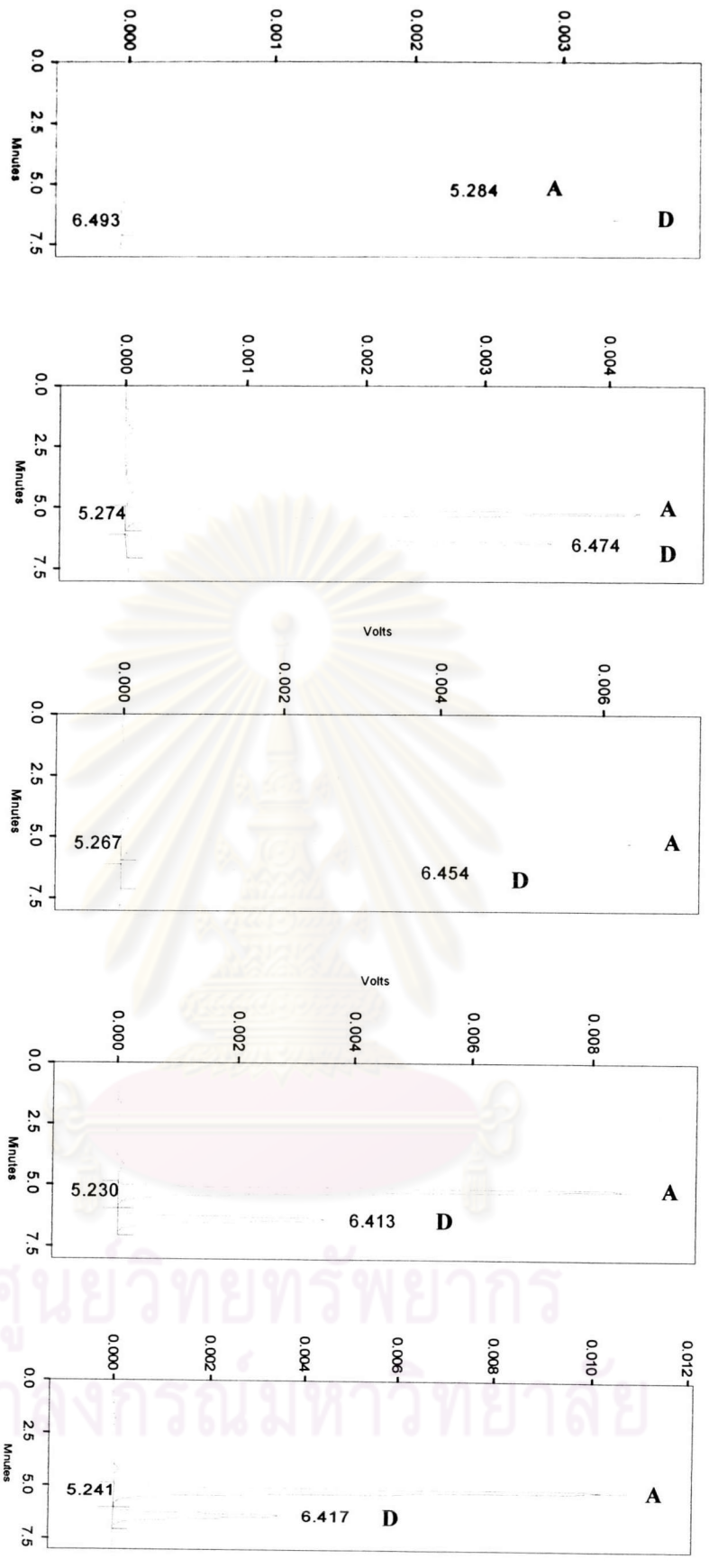


Figure 15A The chromatograms of alprazolam standard solution in various concentrations.

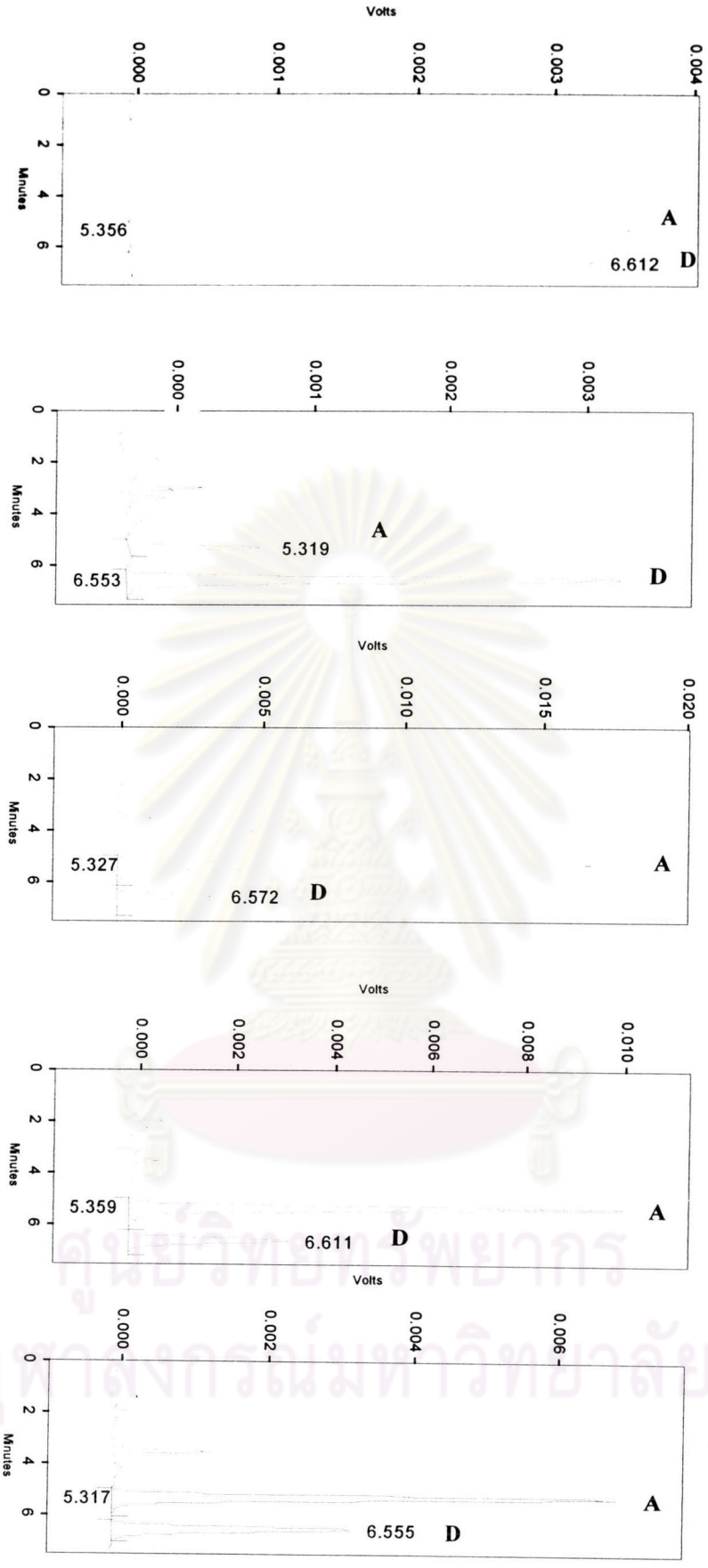


Figure 15AA The chromatograms of alprazolam in which extracted from the submicron emulsion preparation and different phases

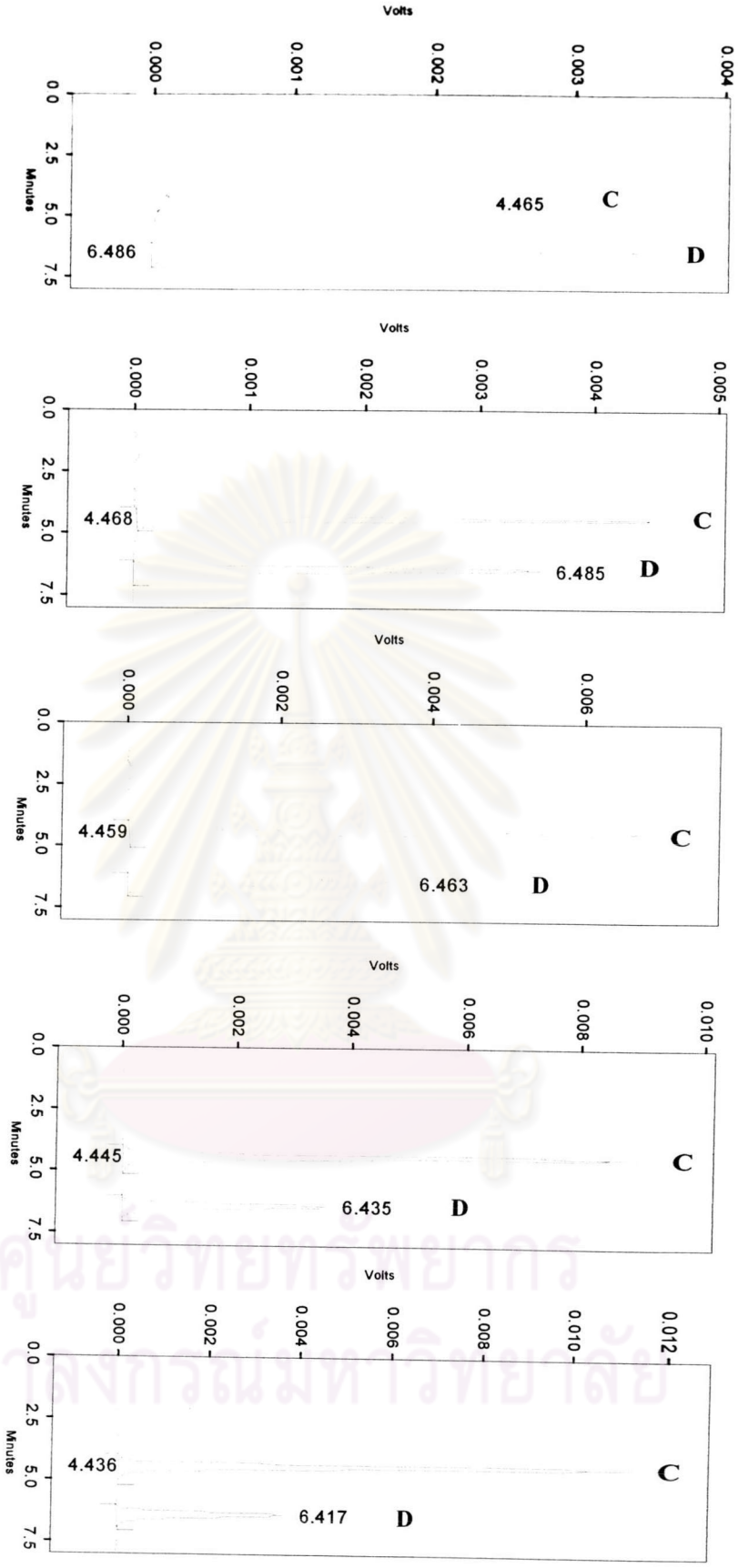


Figure 16A The chromatograms of clonazepam standard solution in various concentrations.

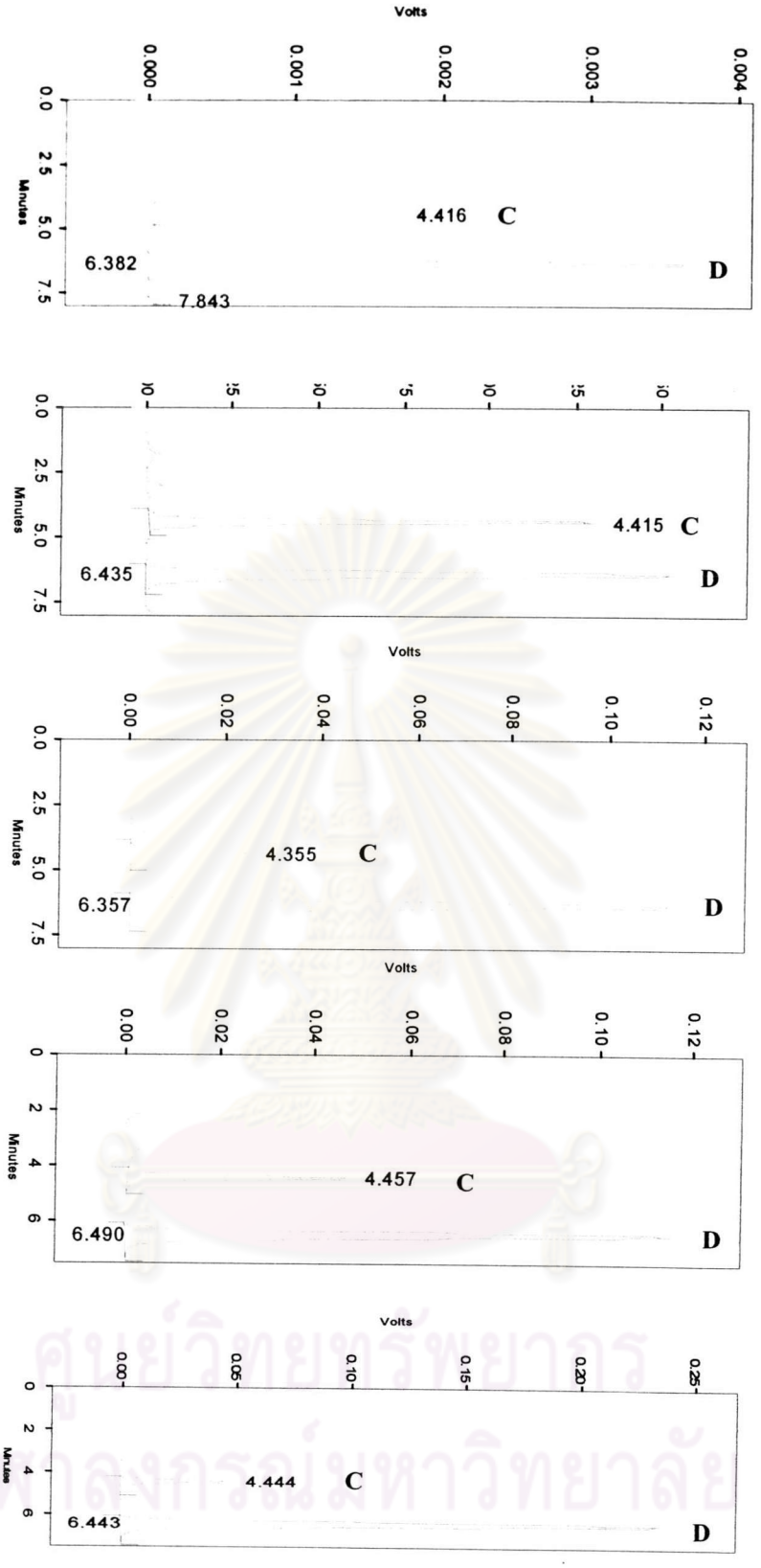


Figure 16AA The chromatograms of clonazepam in which extracted from the submicron emulsion preparation and different phases.

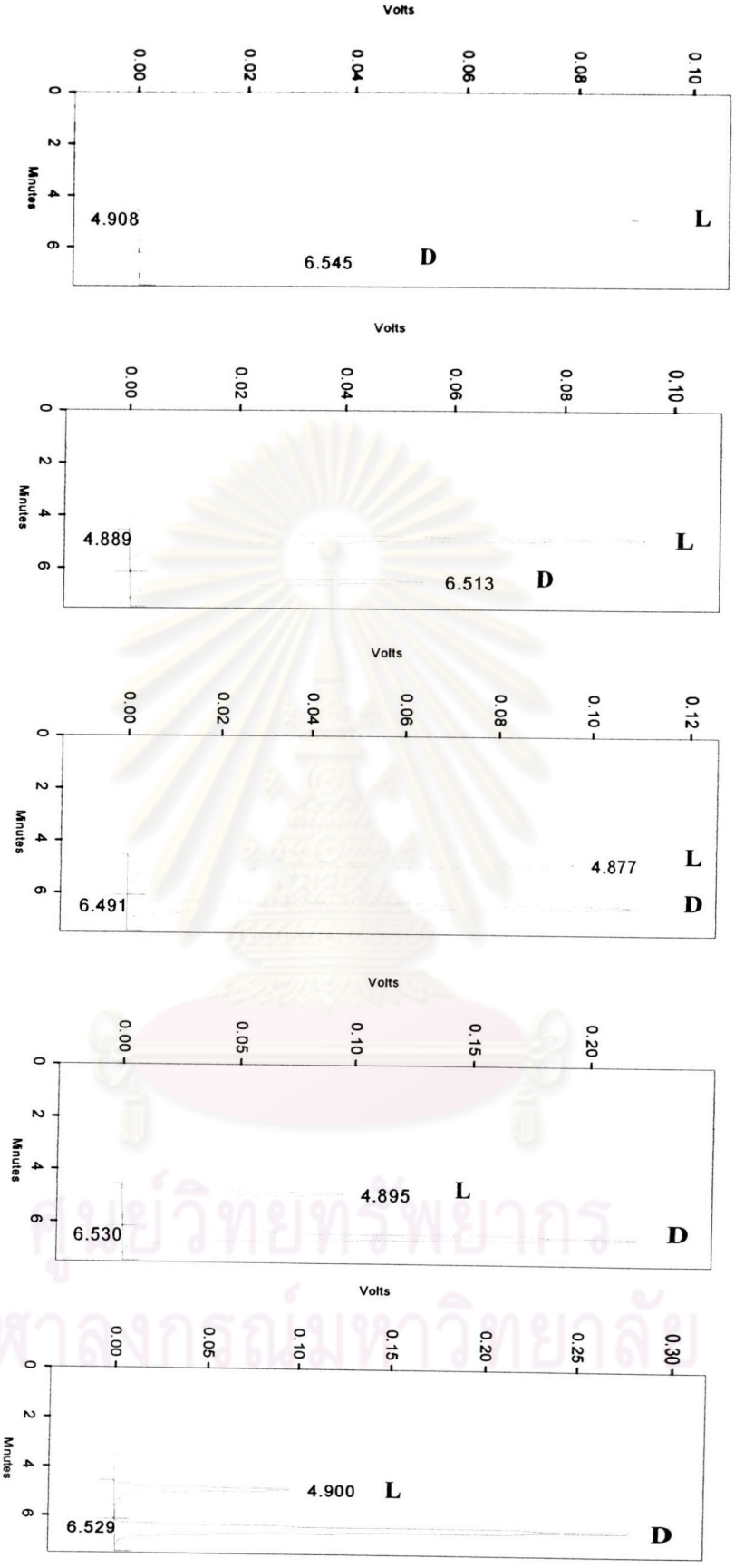


Figure 17A The chromatograms of diazepam standard solution in various concentrations.

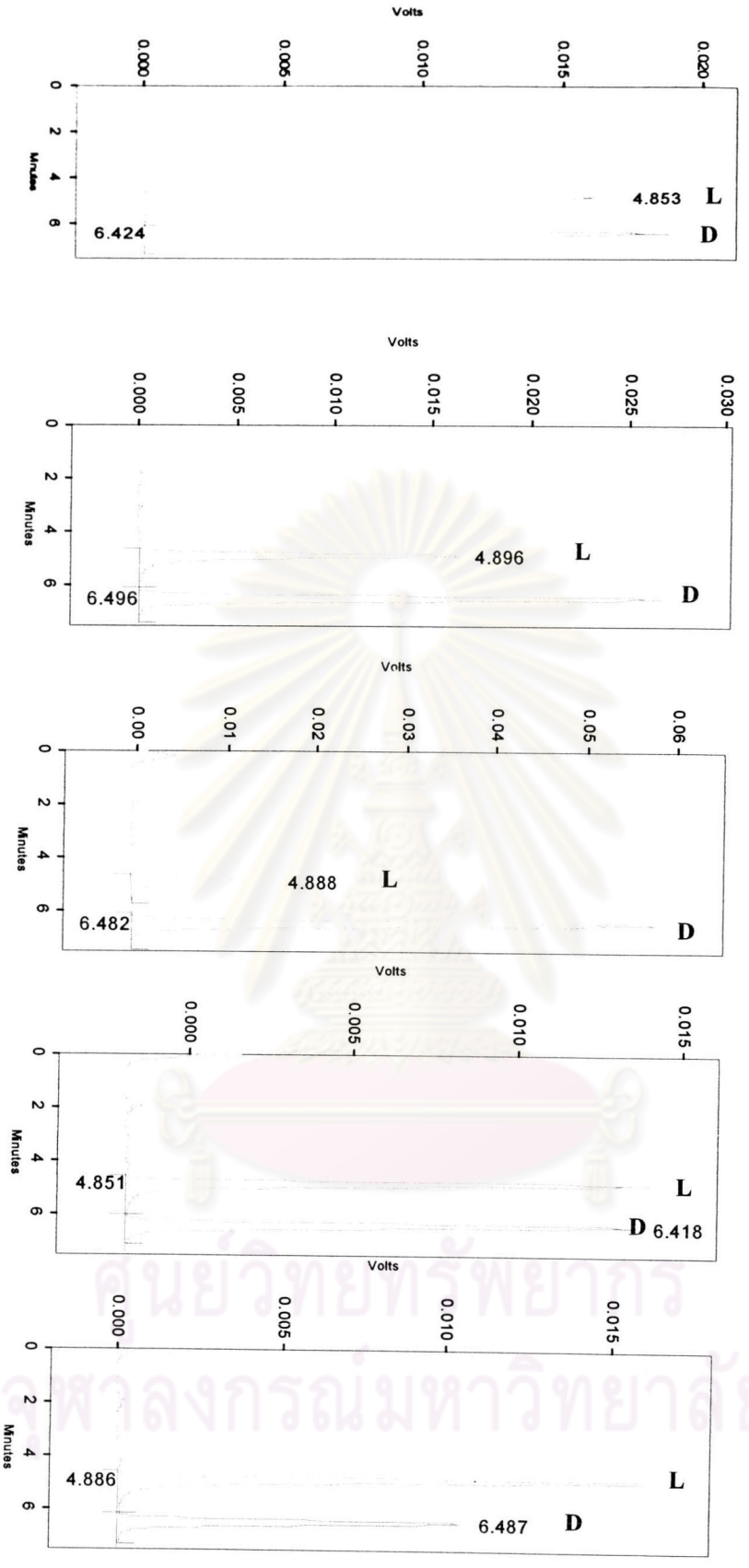


Figure 17AA The chromatograms of diazepam in which extracted from the submicron emulsion preparation and different phases.

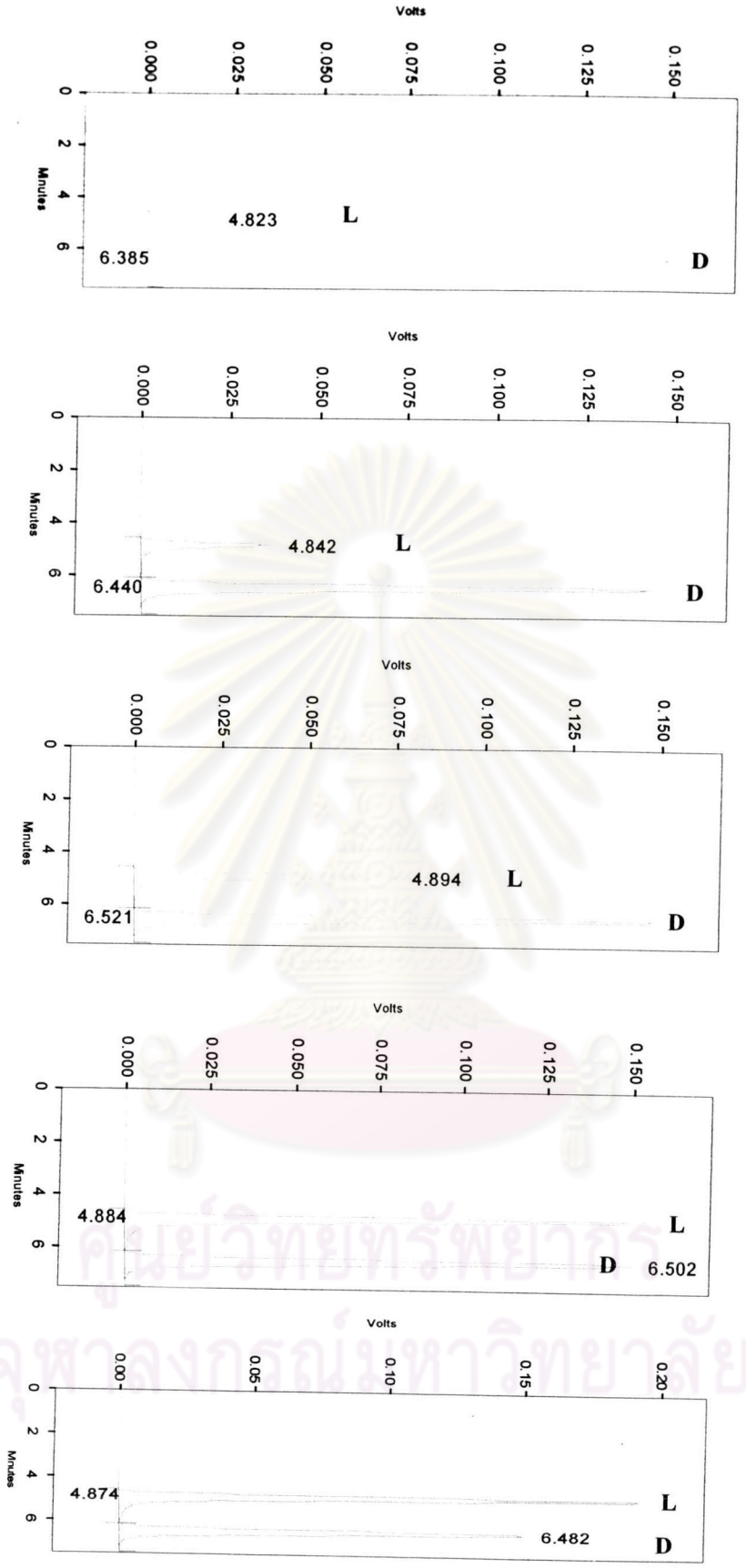


Figure 18A The chromatograms of lorazepam standard solution in various concentrations.

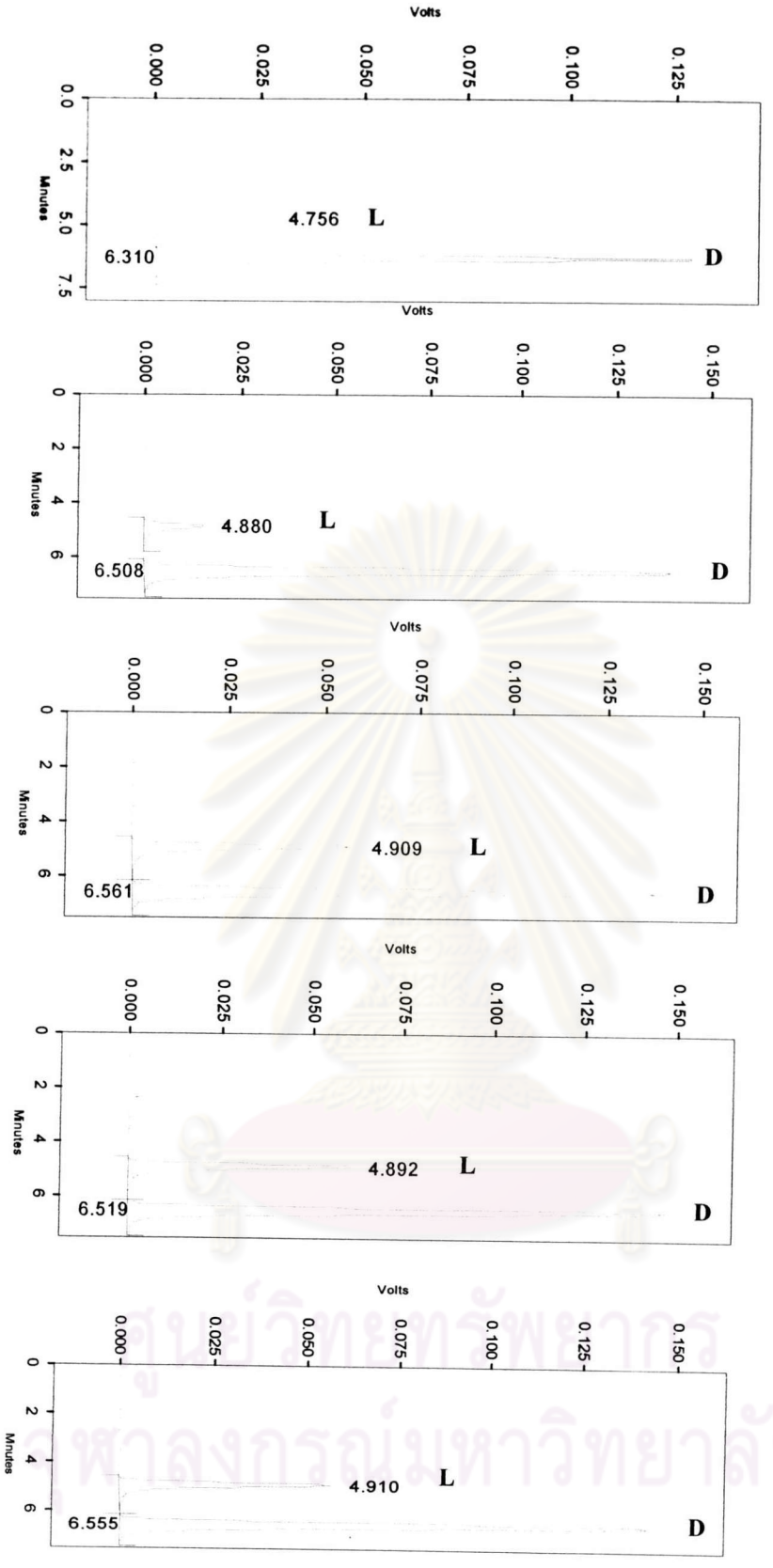


Figure 18AA The chromatograms of lorazepam in which extracted from the submicron emulsion preparation and different phases.

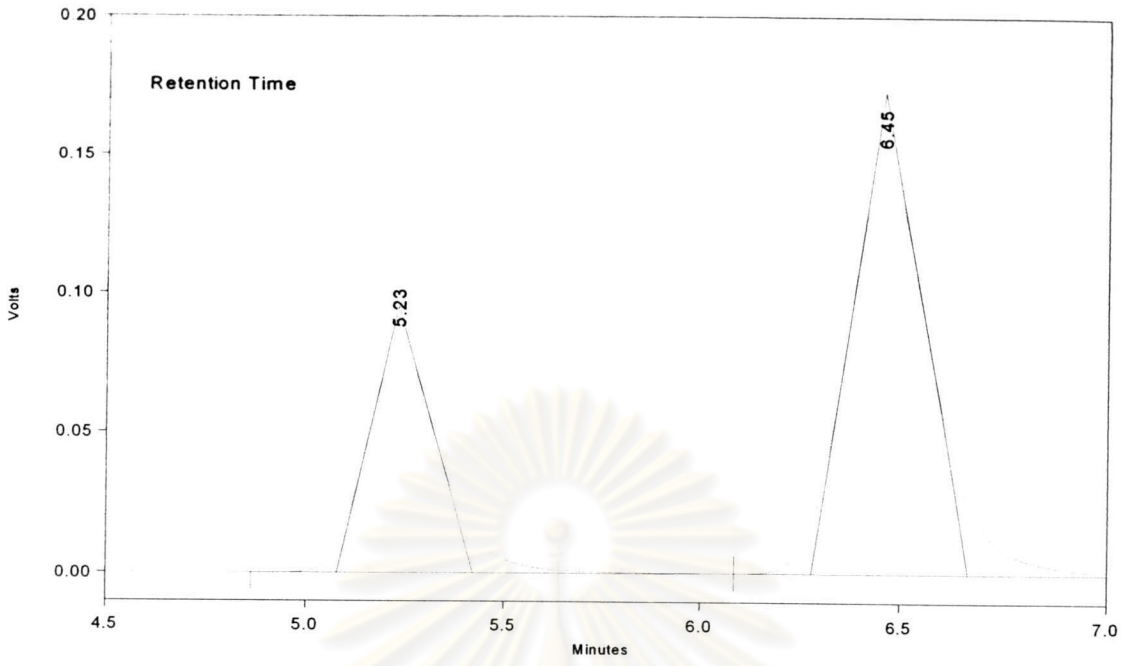


Table 18A Tailing factor and resolution factor of alprazolam and diazepam.

Detector A (254nm) Name	Retention Time	Area	Resolution	Asymmetry	Asymmetry (10%)
alprazolam	5.230	1063754	0.00	1.29	1.22
diazepam	6.455	2249559	3.30	1.24	1.19

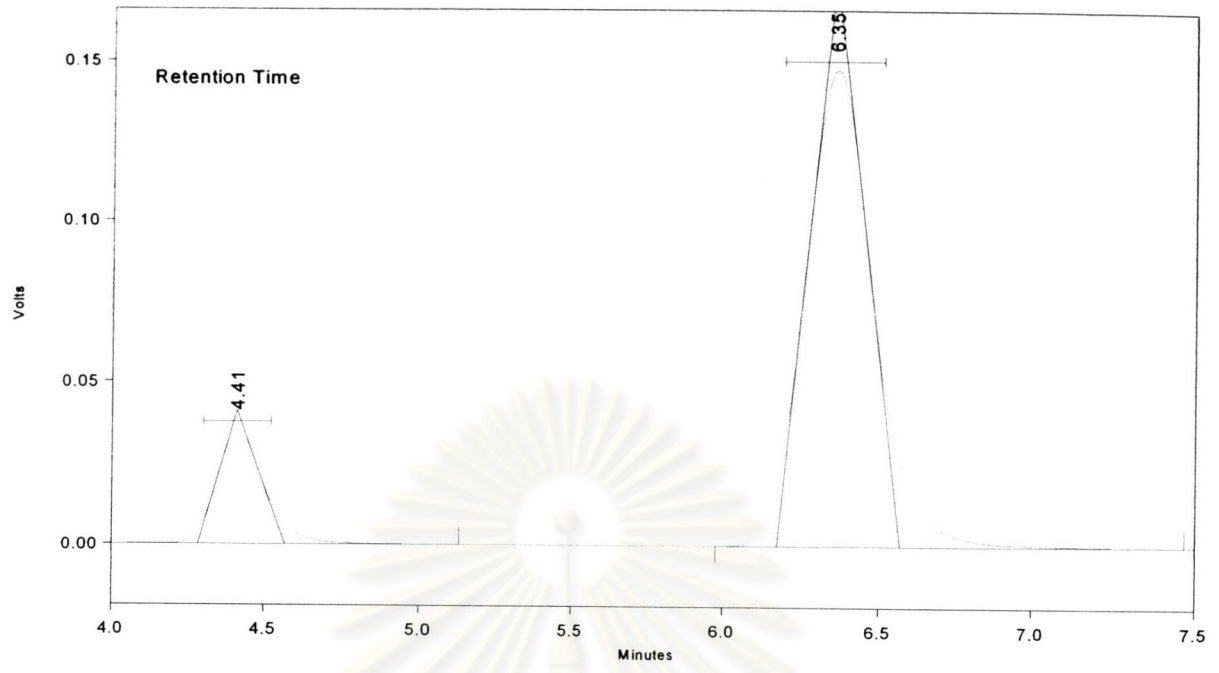


Table 19A Tailing factor and resolution factor of clonazepam and diazepam.

Detector A (254nm) Name	Retention Time	Area	Resolution	Asymmetry	Asymmetry (10%)
clonazepam	4.409	377810	0.00	1.28	1.22
diazepam	6.354	2228913	5.78	1.23	1.18

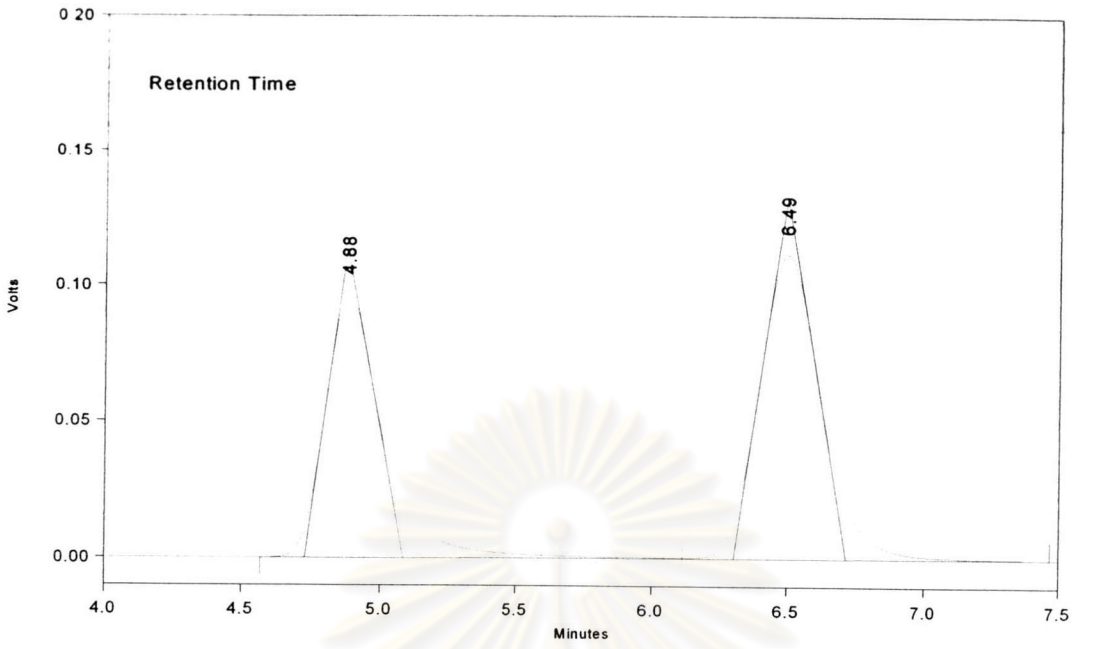


Table 20A Tailing factor and resolution factor of diazepam and clonazepam.

Detector A (254nm) Name	Retention Time	Area	Resolution	Asymmetry	Asymmetry (10%)
lorazepam	4.877	1412819	0.00	1.56	1.40
diazepam	6.491	1743814	4.23	1.24	1.19

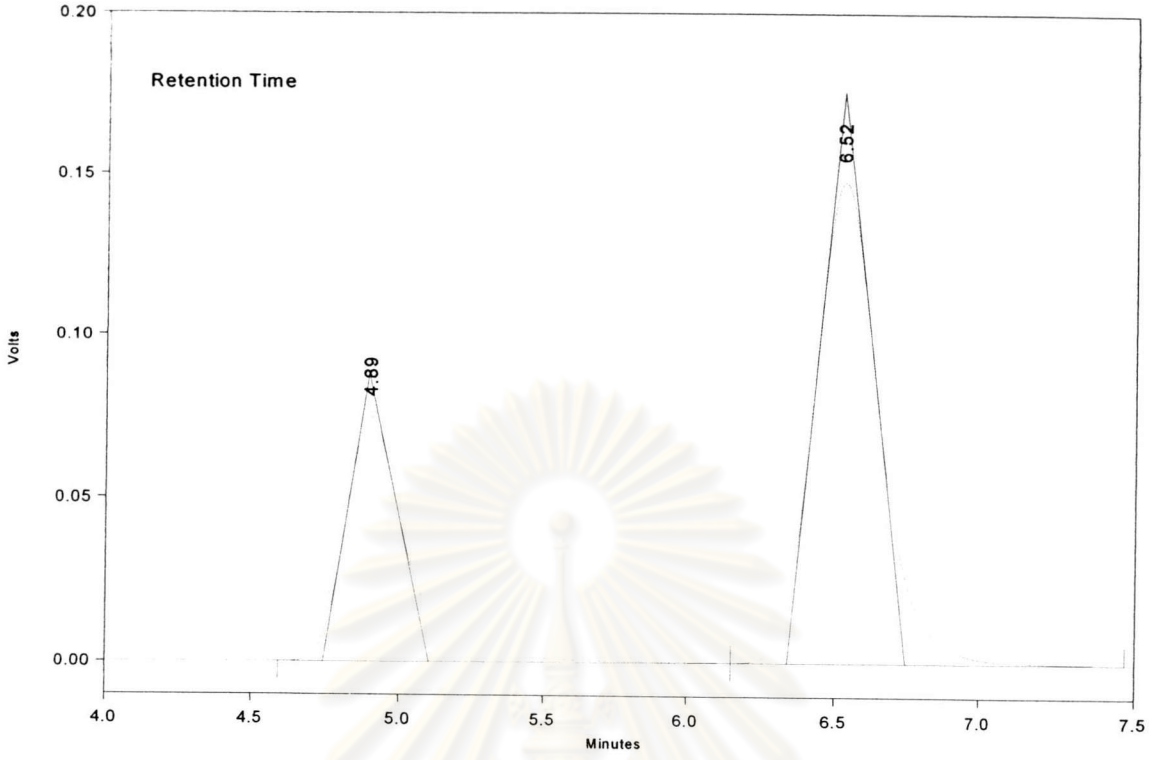


Table 21A Tailing factor and resolution factor of lorazepam and diazepam.

Detector A (254nm) Name	Retention Time	Area	Resolution	Asymmetry	Asymmetry (10%)
lorazepam	4.894	1100432	0.00	1.55	1.39
diazepam	6.521	2307136	4.25	1.24	1.18

Table 22A The repeatability of peak areas of benzodiazepine drugs

Set No	Peak area ratio			
	Alprazolam	Clonazepam	Diazepam	Lorazepam
1	0.5631	0.6134	1.1174	0.5412
2	0.5624	0.6139	1.1188	0.5325
3	0.5638	0.6111	1.1196	0.5416
4	0.5628	0.6174	1.1182	0.5521
5	0.5640	0.6118	1.1182	0.5482
6	0.5633	0.6147	1.1183	0.5443
Average	0.5632	0.6137	1.1184	0.5433
SD	0.0006	0.0022	0.0008	0.0067
%CV	0.1066	0.3673	0.0672	1.2379

Table 23A The recovery of benzodiazepine drugs from soybean oil using solid phase extraction (Extrasep™).

Drug	%Recovery				
	R_1	R_2	Mean	SD	%CV
Alprazolam	104.79	103.95	104.37	0.59	0.56
Clonazepam	91.12	93.92	92.52	1.98	2.14
Diazepam	103.53	108.65	106.09	3.62	3.41
Lorazepam	97.86	96.09	96.98	1.25	1.29

Table 24A The recovery of benzodiazepine drugs from lipid emulsion using solid phase extraction (Extrasep™).

Drug	%Recovery				
	R_1	R_2	Mean	SD	%CV
Alprazolam	101.87	103.37	102.62	1.06	1.03
Clonazepam	104.19	103.27	103.73	0.65	0.63
Diazepam	99.39	99.87	99.63	0.34	0.34
Lorazepam	103.32	103.30	103.31	0.014	0.01

Table 25A The comparison of %recovery of benzodiazepine in various phases between submicron emulsion bases and submicron emulsion containing Arlasolve DMI.

Drug	% Recovery											
	Oil phase			Interface			Aqueous phase			Mesophase		
	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD
Alprazolam	98.98	100.63	99.80±1.17	97.19	97.15	97.17±0.03	102.83	100.08	101.46±1.94	100.59	97.60	99.10±2.11
	93.89	*94.96*	*94.42±0.76*	*95.05*	*96.62*	*95.84±1.11*	*94.92*	*99.98*	*97.45±3.58*	*94.92*	*97.95*	*96.44±2.14*
Clonazepam	80.66	81.60	81.13±0.66	99.01	100.48	99.74±1.04	89.99	92.21	91.10±1.57	100.12	99.39	99.76±0.52
	80.21	*80.79*	*80.50±0.41*	*96.40*	*95.44*	*95.92±0.68*	*93.94*	*97.29*	*95.62±2.37*	*94.41*	*90.18*	*92.30±2.99*
Diazepam	92.43	95.85	94.14±2.42	94.06	96.64	95.35±1.82	101.58	97.74	99.66±2.72	96.12	99.69	97.90±2.52
	96.37	*96.74*	*96.56±0.26*	*101.78*	*96.89*	*99.34±3.46*	*98.32*	*96.51*	*97.42±1.28*	*97.55*	*96.09*	*96.82±1.03*
Lorazepam	90.98	95.48	94.73±1.06	95.75	90.45	93.10±3.75	95.81	99.49	97.65±2.60	91.83	97.17	94.50±3.78
	91.85	*97.22*	*94.54±3.80*	*95.66*	*97.74*	*96.70±1.47*	*92.70*	*98.11*	*95.40±3.82*	*93.05*	*93.69*	*93.37±0.45*

**% recovery of submicron emulsion containg Arlasolve DMI*

Table 26A The precision of alprazolam

Alprazolam concentration (μg/ml)	Calculated concentration from calibration curve (μg/ml)								
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average	SD	%CV
1.136	1.1018	1.1061	1.1025	1.005	1.1054	1.0822	1.0998	0.0088	0.8045
4.544	4.3553	4.3499	4.3606	4.3536	4.3623	4.3569	4.3564	0.0046	0.1050
9.088	9.0841	9.0907	9.0890	9.0816	9.0883	9.0847	9.0864	0.0034	0.0380

Table 27A The precision of clonazepam

Clonazepam concentration (μg/ml)	Calculated concentration from calibration curve (μg/ml)								
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average	SD	%CV
1.04	1.0606	1.0572	1.0629	1.0582	1.0632	1.0605	1.0605	0.0024	0.2283
4.16	4.0839	4.0868	4.0687	4.1101	4.0730	4.0923	4.0858	0.0148	0.3620
8.32	8.4157	8.4128	8.4361	8.4804	8.4794	8.4420	8.4444	0.0297	0.3521

Table 28A The precision of diazepam

Diazepam concentration (μg/ml)	Calculated concentration from calibration curve (μg/ml)								
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average	SD	%CV
1.048	1.0259	1.0190	1.0236	1.0235	1.0202	1.0198	1.0220	0.0027	0.2655
4.192	4.1218	4.1271	4.1300	4.1248	4.1246	4.1250	4.1255	0.0027	0.0666
8.384	8.2293	8.2513	8.2411	8.2349	8.2378	8.2598	8.2424	0.0112	0.1363

Table 29A The precision of lorazepam

Lorazepam concentration (μg/ml)	Calculated concentration from calibration curve (μg/ml)								
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average	SD	%CV
1.064	0.9866	0.9831	0.9791	0.9749	0.9820	0.9844	0.9817	0.0042	0.4237
4.256	4.1858	4.1196	4.1885	4.2689	4.2388	4.2091	4.2018	0.0512	1.2190
8.512	8.4921	8.5022	8.5031	8.5186	8.4894	8.4623	8.4946	0.0189	0.2224

Table 30A The linearity of alprazolam

Alprazolam concentration (μg/ml)	Peak area ratio					
	Set No.1	Set No.2	Set No.3	Average	SD	%CV
0	0	0	0	0	0	0
2.272	0.2744	0.2850	0.2811	0.2802	0.0054	1.9166
4.544	0.5922	0.5917	0.5821	0.5887	0.0057	0.9659
6.816	0.8775	0.8776	0.9007	0.8853	0.0134	1.5134
9.088	1.1879	1.1762	1.1748	1.1796	0.0072	0.6064
11.360	1.4882	1.4884	1.4856	1.4874	0.0015	0.1028

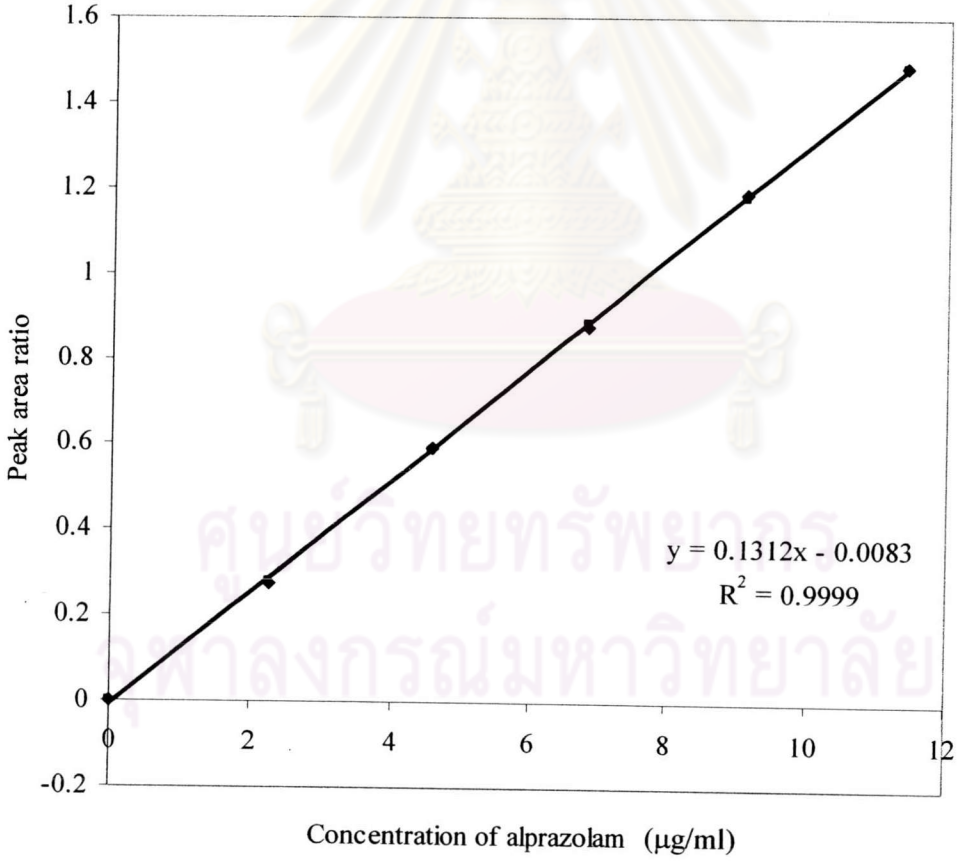


Figure 19A The calibration curve of alprazolam

Table 31A The linearity of clonazepam

Clonazepam concentration (μg/ml)	Peak area ratio					
	Set No.1	Set No.2	Set No.3	Average	SD	%CV
0	0	0	0	0	0	0
2.08	0.2992	0.2975	0.3130	0.3032	0.0085	2.8036
4.16	0.6254	0.6174	0.6275	0.6234	0.0053	0.8494
6.24	0.9211	0.9243	0.9397	0.9284	0.0099	1.0687
8.32	1.2731	1.2598	1.2645	1.2658	0.0067	0.5324
10.40	1.5979	1.5635	1.5795	1.5803	0.0172	1.0905

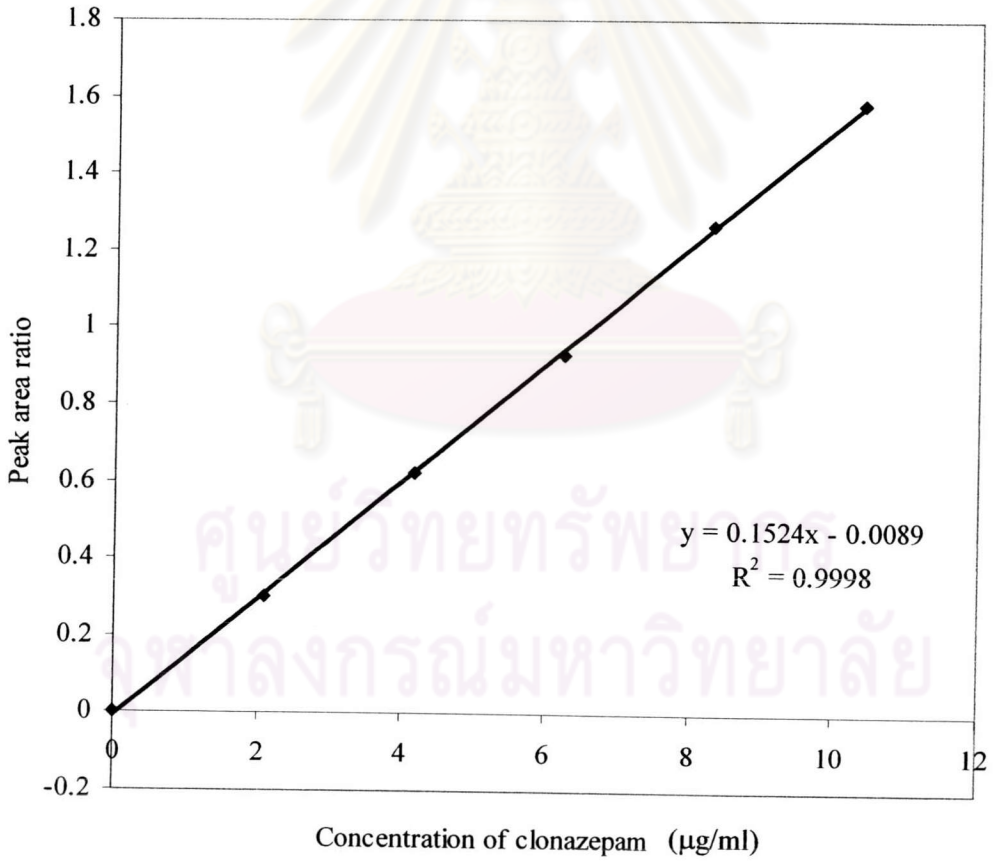


Figure 20A The calibration curve of clonazepam

Table 32A The linearity of diazepam

Diazepam concentration (μg/ml)	Peak area ratio					
	Set No.1	Set No.2	Set No.3	Average	SD	%CV
0	0	0	0	0	0	0
2.096	0.5527	0.5729	0.5603	0.5619	0.0102	1.8148
4.192	1.1593	1.1317	1.1653	1.1521	0.0179	1.5558
6.288	1.6904	1.6748	1.6951	1.6868	0.0106	0.6298
8.384	2.3002	2.2653	2.2774	2.2810	0.0177	0.7775
10.480	2.9154	2.8292	2.8600	2.8682	0.0437	1.5243

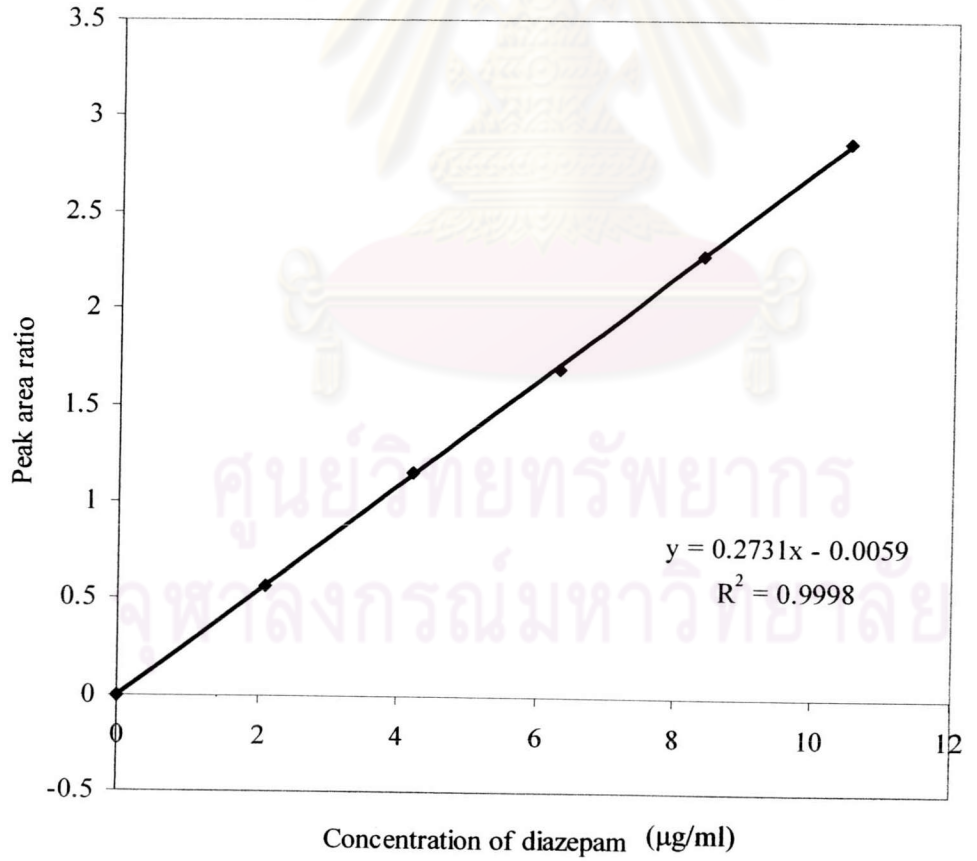


Figure 21A The calibration curve of diazepam

Table 33A The linearity of lorazepam

Lorazepam concentration (μg/ml)	Peak area ratio					
	Set No.1	Set No.2	Set No.3	Average	SD	%CV
0	0	0	0	0	0	0
2.128	0.2685	0.2742	0.2672	0.2700	0.0037	1.3662
4.256	0.5614	0.5398	0.5452	0.5488	0.0112	2.0461
6.384	0.8165	0.8304	0.8171	0.8213	0.0078	0.9556
8.512	1.1240	1.1212	1.0973	1.1142	0.0147	1.3158
10.640	1.3996	1.3822	1.3925	1.3914	0.0088	0.6303

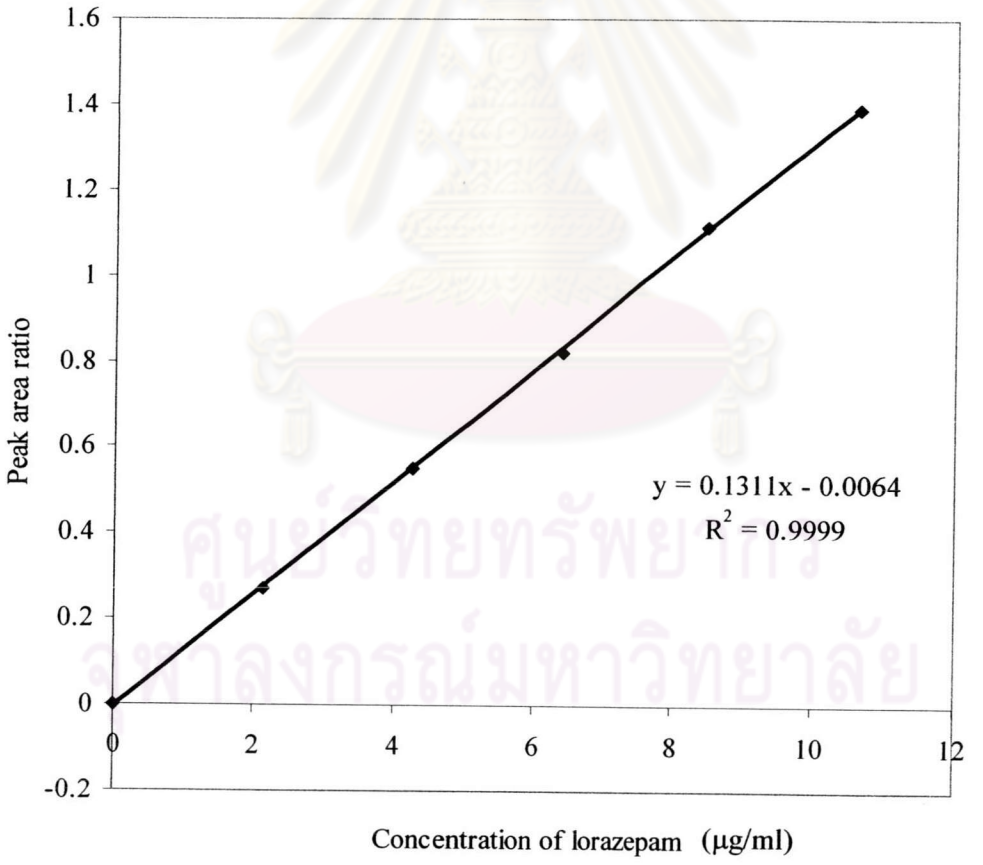


Figure 22A The calibration curve of lorazepam

Table 34A The analytical method validation parameter of HPLC for benzodiazepine drugs.

Parameter	Result value				Limited of acceptability
	Alprazolam	Clonazepam	Diazepam	Lorazepam	
1. System suitability - Tailing factor - Resolution factor - Repeatability of peak area (%CV)	 1.22 3.30 0.11	 1.22 5.78 0.37	 1.19 4.23 0.07	 1.39 4.25 1.24	 ≤2 ≥2 ≤2 [a]
2. Specificity	No other peak interfere	No other peak interfere	No other peak interfere	No other peak interfere	No other peak interfere major peak [a]
3. Accuracy %recovery (SD)	104.37 (0.59)	92.52 (1.98)	106.09 (3.62)	96.98 (1.25)	80-110% [b]
4. Precision (%CV)	0.03-0.80	0.23-0.36	0.06-0.26	0.22-1.21	≤ 2 [b]
5. Linearity -The correlation coefficient (r^2)	0.99986	0.99980	0.99984	0.99988	>0.999 [b]

[a] (The United States Pharmacopeial Convention 2000)

[b] (Jenke 1996)

APPENDIX: PART II ALKYL-4-HYDROXYBENZOATE

DE NOVO EMULSIFICATION

Table 1B The changes in size of paraben submicron emulsions during storage for 7 days at ambient temperature measured by using Photon Correlation Spectroscopy (PCS).

Formulation	Effective mean diameter (nm) (polydispersity)									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Methylparaben Submicron emulsion	252.7 (0.074)	413.4 (0.075)	232.7 (0.123)	201.8 (0.120)	299.5 (0.120)	408.0 (0.116)	281.1 (0.150)	239.6 (0.163)	236.1 (0.143)	307.3 (0.175)
Ethylparaben Submicron emulsion	303.50 (0.107)	332 (0.096)	323.9 (0.239)	372 (0.159)	452.0 (0.258)	501 (0.189)	476.0 (0.260)	511 (0.157)	455.7 (0.164)	499 (0.123)
Propylparaben Submicron emulsion	315.3 (0.163)	407.6 (0.324)	377.6 (0.230)	438.1 (0.054)	435.6 (0.215)	522.3 (0.005)	432.2 (0.169)	462.3 (0.205)	426.0 (0.122)	483.4 (0.173)
Butylparaben Submicron emulsion	435.7 (0.001)	498.3 (0.039)	354.6 (0.129)	381.5 (0.054)	410.7 (0.103)	440.1 (0.083)	434.0 (0.137)	463.3 (0.135)	Could not be prepared	

Table 2B The changes in size of paraben submicron emulsions during storage for 7 days at ambient temperature measured by using laser light diffraction (Mastersizer 2000).

Formulation	D[4,3] mean (nm) (span)									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Methylparaben Submicron emulsion	260 (1.847)	381 (1.194)	560 (1.980)	290 (1.857)	383 (2.031)	463 (1.479)	320 (1.957)	270 (1.791)	330 (1.122)	446 (1.578)
Ethylparaben Submicron emulsion	360 (1.715)	382 (1.279)	2983 (8.117)	5121 (4.102)	1624 (3.902)	2010 (2.157)	841 (2.197)	1113 (2.098)	680 (1.843)	753 (1.361)
Propylparaben Submicron emulsion	458 (2.179)	550 (2.241)	553 (1.989)	380 (2.298)	511 (1.669)	779 (1.264)	482 (1.555)	390 (2.289)	464 (1.472)	400 (2.425)
Butylparaben Submicron emulsion	449 (1.370)	459 (1.513)	361 (1.247)	373 (1.462)	377 (1.281)	408 (1.468)	432 (1.539)	445 (1.541)	Could not be prepared	

Table 3B The changes in zeta potential of paraben submicron emulsions during storage for 7 days at ambient temperature measured by Zetaplus™.

Formulation	Zeta potential mean ±SD									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Methylparaben Submicron emulsion	-27.30 ±1.01	-31.50 ±1.70	-27.70 ±0.70	-28.90 ±1.10	-27.61 ±0.49	-29.87 ±1.72	-19.60 ±1.90	-33.10 ±1.80	-47.70 ±1.90	-67.70 ±2.30
Ethylparaben Submicron emulsion	-22.51 ±2.16	-29.22 ±1.05	-16.71 ±1.30	-22.56 ±0.53	-14.43 ±1.50	-29.51 ±0.87	-17.43 ±0.78	-32.42 ±1.14	-26.20 ±0.46	-42.60 ±2.20
Propylparaben Submicron emulsion	-36.83 ±1.12	-38.26 ±0.92	-37.63 ±1.61	-40.55 ±1.02	-37.70 ±2.27	-42.08 ±1.89	-35.40 ±1.71	-40.21 ±0.84	-37.20 ±2.18	-41.10 ±2.01
Butylparaben Submicron emulsion	-30.93 ±1.30	-20.14 ±2.07	-38.89 ±1.65	-20.21 ±2.68	-35.52 ±1.12	-18.55 ±2.76	-33.79 ±1.57	-26.36 ±2.71	Could not be prepared	

Table 4B The changes in pH of paraben submicron emulsions during storage for 7 days at ambient temperature.

Formulation	pH mean ± SD									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Methylparaben Submicron emulsion	5.37 ±0.01	4.84 ±0.01	6.01 ±0.01	5.87 ±0.02	5.02 ±0.02	5.44 ±0.01	5.87 ±0.02	5.53 ±0.02	5.56 ±0.01	5.40 ±0.02
Ethylparaben Submicron emulsion	5.36 ±0.01	5.26 ±0.01	5.54 ±0.01	5.28 ±0.02	5.31 ±0.01	5.14 ±0.01	5.15 ±0.01	5.40 ±0.08	5.23 ±0.02	5.81 ±0.07
Propylparaben Submicron emulsion	5.70 ±0.01	5.20 ±0.01	5.41 ±0.02	5.06 ±0.01	5.97 ±0.02	5.48 ±0.01	5.43 ±0.01	5.20 ±0.01	5.31 ±0.01	5.10 ±0.01
Butylparaben Submicron emulsion	5.13 ±0.06	4.87 ±0.02	5.52 ±0.01	5.28 ±0.01	5.90 ±0.01	5.71 ±0.02	5.89 ±0.01	5.55 ±0.01	Could not be prepared	

Table 5B %Recovery content of parabens from various concentration of submicron emulsions prepared by de novo method.

Concentration (%)	Methylparaben			Ethylparaben			Propylparaben			Butylparaben		
	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD
30	85.22	91.26	88.24±4.26	74.04	74.72	74.38±0.48	106.86	106.55	106.70±0.22	102.48	101.03	101.76±1.03
40	96.85	112.99	104.92±11.41	73.25	75.99	74.62±1.94	97.86	96.71	97.28±0.81	97.14	98.63	97.88±1.05
50	80.20	83.82	82.01±2.56	87.54	87.72	87.48±0.09	115.73	114.86	115.29±0.62	103.26	101.66	102.46±1.14
60	114.89	112.12	113.50±1.96	90.80	87.22	89.01±2.53	113.47	109.64	111.56±2.71	95.06	96.70	95.88±1.15
70	106.62	106.32	106.47±0.21	112.54	112.64	112.59±0.07	112.40	114.28	113.34±1.33	-	-	-

Table 6B % Weight loss after separating by ultracentrifugation of methylparaben submicron emulsions prepared by de novo emulsification.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Lose weight (g)	% loss	Mean±SD
30%	1	0.4665	0.1781	5.9489	0.3872	6.9807	7.1326	0.1519	2.13	1.98±0.25
	2	0.464	0.2323	5.9244	0.3867	7.0074	7.1596	0.1522	2.12	
	3	0.5335	0.2049	5.8941	0.3807	7.0132	7.1335	0.1203	1.69	
40%	1	0.5466	0.2686	5.8722	0.3156	7.0030	7.1146	0.1116	1.57	1.15±0.36
	2	0.5191	0.2336	5.9261	0.2733	6.9521	7.0172	0.0651	0.93	
	3	0.5827	0.2439	5.8913	0.2766	6.9945	7.0616	0.0671	0.95	
50%	1	0.4526	0.1674	5.8798	0.3793	6.8791	7.1345	0.2554	3.58	2.05±1.32
	2	0.5403	0.1633	5.9674	0.3957	7.0667	7.1553	0.0886	1.24	
	3	0.4752	0.1665	6.0172	0.3851	7.0440	7.1394	0.0954	1.34	
60%	1	0.6166	0.1359	5.8404	0.3995	6.9924	7.0861	0.0937	1.32	1.74±0.98
	2	0.6110	0.2146	5.6537	0.3492	6.8285	7.0306	0.2021	2.87	
	3	0.6247	0.1285	5.8029	0.4051	6.9612	7.0346	0.0734	1.04	
70%	1	0.6864	0.1268	5.7906	0.3577	6.9615	7.0392	0.0777	1.10	0.96±0.13
	2	0.5625	0.2050	5.8485	0.3537	6.9697	7.0292	0.0595	0.85	
	3	0.5865	0.1452	5.9121	0.3236	6.9674	7.0324	0.0650	0.92	

Table 7B %Weight loss after separating by ultracentrifugation of ethylparaben submicron emulsions prepared by de novo emulsification.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Lose weight (g)	% loss	Mean±SD
30%	1	0.3210	0.1842	6.0084	0.4044	6.9180	7.0031	0.0851	1.22	2.13±0.79
	2	0.4040	0.1280	5.9054	0.3881	6.8255	7.0063	0.1808	2.58	
	3	0.5088	0.2059	5.6368	0.4718	6.8233	7.0051	0.1818	2.60	
40%	1	0.5382	0.1785	5.8894	0.3139	6.92	7.0081	0.0881	1.26	2.00±0.64
	2	0.5586	0.1778	5.7940	0.3061	6.8365	7.0025	0.1660	2.37	
	3	0.5694	0.2046	5.7443	0.3213	6.8396	7.0047	0.1651	2.36	
50%	1	0.8082	0.1646	5.5267	0.3182	6.8177	7.0081	0.1904	2.72	2.10±0.87
	2	-	-	-	-	-	-	-	-	
	3	0.6024	0.2299	5.7047	0.3598	6.8968	7.0009	0.1041	1.49	
60%	1	0.6127	0.1713	5.6650	0.3815	6.8305	7.0006	0.1701	2.43	2.29±0.86
	2	0.6461	0.2058	5.7398	0.3136	6.9053	7.0002	0.0949	1.36	
	3	0.7147	0.1820	5.6010	0.2883	6.7860	7.0007	0.2147	3.07	
70%	1	0.5022	0.2587	5.8313	0.3086	6.9008	7.0009	0.1001	1.43	1.67±0.59
	2	0.8291	0.2914	5.4852	0.3085	6.9142	7.0011	0.0869	1.24	
	3	0.9003	0.2535	5.3778	0.3050	6.8366	7.0003	0.1637	2.34	

Table 8B %Weight loss after separating by ultracentrifugation of propylparaben submicron emulsions prepared by de novo emulsification.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Lose weight (g)	% loss	Mean±SD
30%	1	0.5974	0.2916	5.7152	0.3452	6.9494	7.1942	0.2448	3.40	3.66±0.98
	2	0.6582	0.2224	5.7559	0.3300	6.9655	7.1702	0.2037	2.84	
	3	0.6469	0.2456	5.6384	0.2990	6.8299	7.1701	0.3402	4.74	
40%	1	0.5744	0.1820	5.7491	0.4177	6.9232	7.1823	0.2591	3.61	2.71±0.80
	2	0.5243	0.1753	5.8333	0.5070	7.0399	7.1904	0.1505	2.09	
	3	0.6185	0.0962	5.7529	0.5512	7.0188	7.1940	0.1752	2.44	
50%	1	0.1642	0.1840	6.2185	0.2165	6.7832	7.0020	0.2188	3.12	2.28±0.73
	2	0.5065	0.1774	5.9585	0.2369	6.8793	7.0016	0.1223	1.75	
	3	0.5126	0.2012	5.9095	0.2583	6.8816	7.0205	0.1389	1.98	
60%	1	0.1161	0.0965	6.3746	0.2949	6.8821	7.0104	0.1283	1.83	2.15±0.32
	2	0.4933	0.1236	6.0740	0.1992	6.8901	7.0410	0.1509	2.14	
	3	0.5618	0.1223	5.8097	0.3562	6.8500	7.0234	0.1734	2.47	
70%	1	0.3001	0.1165	5.9359	0.3211	6.6736	7.0225	0.3489	4.97	3.85±0.97
	2	0.6106	0.2060	5.6702	0.3007	6.7875	7.0168	0.2293	3.27	
	3	0.8310	0.0660	5.4048	0.6463	6.9481	7.1850	0.2369	3.30	

Table 9B %Weight loss after separating by ultracentrifugation of buthylparaben submicron emulsions prepared by de novo emulsification.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Lose weight (g)	% loss	Mean±SD
30%	1	0.7183	0.1851	5.6910	0.4751	7.0695	7.1385	0.0690	0.97	2.20±1.60
	2	0.5448	0.2872	5.7307	0.3381	6.9008	7.0146	0.1138	1.62	
	3	0.6484	0.1871	5.5648	0.3306	6.7309	7.0123	0.2814	4.01	
40%	1	0.5328	0.2524	5.6183	0.4928	6.8963	7.0061	0.1098	1.57	1.77±1.03
	2	0.5860	0.0560	5.6182	0.5521	6.8123	7.0151	0.2028	2.89	
	3	0.6719	0.0283	5.7340	0.5210	6.9552	7.0146	0.0594	0.85	
50%	1	0.5094	0.0839	6.0996	0.3503	7.0432	7.1369	0.0937	1.31	2.27±1.49
	2	0.6519	0.0935	5.7468	0.3601	6.8523	7.1373	0.2850	3.99	
	3	0.5200	0.0695	6.0991	0.3390	7.0276	7.1360	0.1084	1.52	
60%	1	0.5841	0.1163	6.0239	0.3614	7.0857	7.1387	0.0530	0.74	1.88±1.56
	2	0.6366	0.1129	5.9414	0.3552	7.0461	7.1341	0.0880	1.23	
	3	0.6120	0.1455	5.7615	0.3584	6.8774	7.1384	0.2610	3.66	

Table 10B The analytical amount and recovery after ultracentrifugation of methylparaben submicron emulsion prepared by de novo emulsification.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0.3850	0.5206	0.9249	0.4643	2.2949	7.1326	2.9346	78.20	77.14 ±2.94
	2	0.3736	0.5064	0.9510	0.5078	2.3388	7.1596	2.9457	79.40	
	3	0.3820	0.4330	0.9627	0.3885	2.1663	7.1335	2.9349	73.81	
40%	1	0.5717	0.6540	1.3266	0.9867	3.5390	7.1146	3.9232	90.21	95.49 ±4.63
	2	0.5802	0.7904	1.3800	1.0188	3.7695	7.0172	3.8695	97.42	
	3	0.6682	0.7958	1.3353	1.0501	3.8494	7.0616	3.8940	98.86	
50%	1	0.6338	0.4279	1.7261	0.9139	3.7018	7.1345	4.8922	75.67	73.37 ±8.60
	2	0.7380	0.2868	1.6355	0.4727	3.1330	7.1553	4.9065	63.85	
	3	0.6689	0.3588	1.9561	0.9614	3.9452	7.1394	4.8956	80.59	
60%	1	1.0224	0.5308	2.0330	1.9035	5.4898	7.0861	5.8410	93.99	96.18 ±1.96
	2	1.0570	0.8872	1.9449	1.7774	5.6665	7.0306	5.7952	97.78	
	3	1.0289	0.5930	2.1600	1.8099	5.5918	7.0346	5.7784	96.77	
70%	1	1.1628	0.5585	2.1103	1.8835	5.7150	7.0392	6.7375	84.82	93.71 ±11.12
	2	2.1342	0.8944	2.1820	1.9335	7.1440	7.0292	6.7279	106.18	
	3	1.0801	0.7210	2.5145	1.7507	6.0663	7.0324	6.7310	90.12	

Table 11B The analytical amount and recovery after ultracentrifugation of ethylparaben submicron emulsion prepared by de novo emulsification.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0.3899	0.4566	0.7682	0.9814	2.5961	7.0031	4.0118	64.71	65.40 ±1.46
	2	0.5566	0.2656	0.8226	0.9402	2.5850	7.0063	4.0136	64.41	
	3	0.5515	0.4689	0.6836	0.9878	2.6918	7.0051	4.0129	67.08	
40%	1	0.7701	0.6334	1.0423	1.3945	3.8404	7.0081	5.2461	73.20	70.36 ±2.57
	2	0.8382	0.7418	0.8759	1.1947	3.6506	7.0025	5.2419	69.64	
	3	0.6603	0.6575	0.9701	1.2891	3.5771	7.0047	5.2435	68.22	
50%	1	1.3882	0.8635	1.6720	1.4176	5.3414	7.0081	6.6076	80.84	77.28 ±5.02
	2	-	-	-	-	-	-	-	-	
	3	1.4270	1.0291	1.0404	1.3706	4.8671	7.0009	6.6008	73.73	
60%	1	2.1273	1.1391	1.2444	1.6454	6.1562	7.0006	7.8907	78.02	75.29 ±2.64
	2	1.9016	1.3578	1.2598	1.4067	5.9259	7.0002	7.8902	75.10	
	3	1.8961	1.1499	1.4996	1.1954	5.7410	7.0007	7.8908	72.76	
70%	1	1.8062	2.5306	1.6671	1.4049	7.4087	7.0009	9.2412	80.17	82.56 ±2.63
	2	2.4882	2.3252	1.6572	1.4193	7.8898	7.0011	9.2414	85.37	
	3	2.4535	1.9533	1.5968	1.5854	7.5890	7.0003	9.2404	82.13	

Table 12B The analytical amount and recovery after ultracentrifugation of propylparaben submicron emulsion prepared by de novo emulsification.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	1.3958	1..7144	1.1348	1.2550	5.4999	7.1942	5.8992	93.23	93.50 ±1.73
	2	1.6647	1.4826	1.1780	1.2805	5.6059	7.1702	5.8796	95.34	
	3	1.6636	1.4307	1.1174	1.1923	5.4039	7.1701	5.8795	91.91	
40%	1	2.0564	1.2595	1.3329	2.0521	6.7009	7.1823	7.9005	84.82	86.47 ±1.90
	2	1.9178	1.3092	1.2051	2.5714	7.0035	7.1904	7.9094	88.55	
	3	1.8586	0.9577	1.5174	2.4748	6.8087	7.1940	7.9134	86.04	
50%	1	0.6476	2.2605	1.1293	1.3616	5.3990	7.0020	9.6628	55.87	71.37 ±15.32
	2	1.5074	2.8391	1.2429	1.3421	6.9314	7.0016	9.6622	71.74	
	3	2.1916	3.1380	1.4291	1.6223	8.3810	7.0205	9.6883	86.51	
60%	1	0.7550	1.5074	1.4841	2.7005	6.4470	7.0104	11.6072	55.54	69.65 ±12.82
	2	1.9296	2.0426	2.7794	1.7356	8.4873	7.0410	11.6579	72.80	
	3	2.5934	1.8733	1.9256	2.9809	9.3732	7.0234	11.6287	80.60	
70%	1	1.2617	1.1397	1.4167	3.5308	7.3490	7.0225	13.4732	54.54	75.04 ±20.35
	2	2.4921	2.9681	1.5130	3.1776	10.1508	7.0168	13.4723	75.34	
	3	4.2684	0.4054	0.9142	7.5405	13.1285	7.1850	13.7849	95.24	

Table 13B The analytical amount and recovery after ultracentrifugation of buthylparaben submicron emulsion prepared by de novo emulsification.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	2.3684	1.9114	1.7399	2.6644	8.6842	7.1385	12.0131	72.29	79.91 ±0.91
	2	3.2217	2.9580	0.8416	1.7097	8.7309	7.0146	11.8046	73.96	
	3	3.8447	1.7044	1.1845	1.8210	8.5546	7.0123	11.8007	72.49	
40%	1	3.9849	1.3612	0.8178	3.7323	9.8963	7.0061	15.7237	62.94	67.21 ±3.88
	2	4.9297	0.3590	0.6289	4.8120	10.7296	7.0151	15.7439	68.15	
	3	5.1078	0.2252	0.7279	5.0421	11.1031	7.0146	15.7428	70.53	
50%	1	4.6871	0.7513	0.9351	6.0088	12.3823	7.1369	19.9833	61.96	63.44 ±3.99
	2	4.3131	0.6099	1.1034	6.0440	12.0704	7.1373	19.9844	60.40	
	3	4.3136	0.8044	1.6778	6.7832	13.5790	7.1360	19.9808	67.96	
60%	1	5.5540	1.4667	1.3822	6.8468	15.2497	7.1387	23.9554	63.66	62.64 ±4.71
	2	5.3645	1.4178	1.7050	5.2768	13.7640	7.1341	23.9400	57.49	
	3	5.1213	1.6663	1.0417	8.1615	15.9909	7.1384	23.9544	66.76	

Table 14B Percentage of drug distribution into various phases of submicron emulsion prepared by de novo emulsification.

%drug satutared	%drug distribution (Mean±SD)															
	Oil phase				PC rich phase				Aqueous phase				Mesophase			
	MP	EP	PP	BP	MP	EP	PP	BP	MP	EP	PP	BP	MP	EP	PP	BP
30%	16.80 ±0.83	19.01 ±3.50	28.62 ±2.86	36.37 ±8.85	21.44 ±1.36	15.09 ±4.17	28.03 ±2.72	25.27 ±7.53	41.80 ±2.29	28.94 ±3.26	20.77 ±0.21	14.51 ±5.23	19.96 ±1.90	36.96 ±0.75	22.57 ±0.44	23.85 ±5.98
40%	16.30 ±0.99	20.49 ±2.28	28.66 ±1.63	44.07 ±3.29	20.04 ±1.36	18.40 ±1.91	16.25 ±2.89	6.38 ±0.93	36.26 ±1.43	26.08 ±1.81	22.39 ±5.58	6.89 ±1.24	27.40 ±0.44	35.02 ±2.00	32.70 ±4.65	42.66 ±4.29
50%	19.21 ±3.76	27.65 ±2.35	19.96 ±7.24	35.12 ±3.09	9.94 ±1.41	18.66 ±3.52	40.09 ±2.34	5.68 ±0.55	49.47 ±2.79	26.34 ±7.02	18.63 ±2.03	9.68 ±2.45	21.38 ±5.45	27.35 ±1.14	21.31 ±3.38	49.52 ±0.86
60%	18.56 ±0.14	33.22 ±1.24	20.70 ±8.17	35.81 ±3.51	11.98 ±3.22	20.48 ±2.24	22.48 ±2.18	10.11 ±0.43	36.66 ±2.18	22.53 ±3.15	25.44 ±6.45	9.32 ±2.94	32.80 ±1.69	23.76 ±2.95	31.38 ±10.72	44.76 ±6.35
70%	22.67 ±6.36	29.41 ±4.38	24.74 ±7.67	-	11.39 ±1.44	29.79 ±4.22	15.94 ±3.09	-	36.31 ±5.48	21.52 ±0.85	16.71 ±6.24	-	29.63 ±3.02	19.28 ±1.48	45.49 ±13.24	-

APPENDIX: PART II ALKYL-4-HYDROXYBENZOATE

EXTEMPORANEOUS ADDITION

Table 15BThe change in size of paraben submicron emulsions (nm) containing Arlasolve DMI during storage for 7 days at ambient temperature measured by using Photon Correlation Spectroscopy (PCS).

Formulation	Effective diameter (nm)(polydispersity)									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Emulsion base	267.8 (0.088)	270.5 (0.099)	267.8 (0.088)	270.5 (0.099)	288.9 (0.131)	290.3 (0.156)	288.9 (0.131)	290.3 (0.156)	288.9 (0.131)	290.3 (0.156)
Methylparaben	267.7 (0.091)	273.0 (0.118)	271.3 (0.071)	269.7 (0.088)	295.2 (0.125)	301.8 (0.140)	295.0 (0.103)	302.1 (0.117)	298.1 (0.147)	303.4 (0.143)
Emulsion base	262.8 (0.104)	277.9 (0.068)	262.8 (0.104)	277.9 (0.068)	262.8 (0.104)	277.9 (0.068)	248.3 (0.110)	259.1 (0.074)	248.3 (0.110)	259.1 (0.074)
Ethylparaben	254.7 (0.115)	294.8 (0.250)	265.8 (0.128)	349.5 (0.135)	281.2 (0.134)	315.2 (0.171)	267.8 (0.042)	290.8 (0.127)	280.6 (0.073)	299.7 (0.118)
Emulsion base	264.9 (0.105)	272.7 (0.095)	264.9 (0.105)	272.7 (0.095)	264.9 (0.105)	272.7 (0.095)	264.9 (0.105)	272.7 (0.095)	267.8 (0.088)	270.5 (0.099)
Propylparaben	287.6 (0.111)	309.3 (0.181)	297.6 (0.109)	329.5 (0.123)	323.2 (0.116)	347.1 (0.072)	330.6 (0.136)	356.3 (0.219)	322.3 (0.045)	370.4 (0.026)

Table 16B The change in size of paraben submicron emulsions (nm) containing Arlasolve DMI during storage for 7 days at ambient temperature measured by using laser light diffraction (Mastersizer 2000).

Formulation	D[4,3] mean (nm) (span)									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Emulsion base	255 (1.486)	265 (1.099)	255 (1.486)	265 (1.099)	365 (1.567)	403 (1.512)	365 (1.567)	403 (1.512)	365 (1.567)	403 (1.512)
Methylparaben	285 (1.597)	284 (1.562)	294 (1.602)	296 (1.601)	381 (1.783)	437 (1.909)	359 (1.682)	381 (1.697)	373 (1.674)	453 (1.785)
Emulsion base	262 (1.449)	340 (1.334)	262 (1.449)	340 (1.334)	262 (1.449)	340 (1.334)	237 (1.515)	280 (1.538)	237 (1.515)	280 (1.538)
Ethylparaben	286 (1.498)	412 (1.582)	286 (1.514)	440 (1.502)	289 (1.527)	603 (2.283)	260 (1.590)	381 (1.653)	272 (1.680)	432 (2.361)
Emulsion base	271 (1.414)	270 (1.396)	271 (1.414)	270 (1.396)	271 (1.414)	270 (1.396)	271 (1.414)	270 (1.396)	255 (1.486)	265 (1.099)
Propylparaben	297 (1.615)	344 (1.482)	327 (1.650)	356 (1.333)	379 (1.756)	397 (1.605)	388 (1.705)	444 (1.420)	374 (1.474)	388 (1.409)

Table 17B The change in zeta potential of paraben submicron emulsions containing Arlasolve DMI during storage for 7 days at ambient temperature measured by Zetaplus™.

Formulation	Zeta Potential±SD									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Emulsion base	-22.09 ±3.63	-25.81 ±4.07	-22.09 ±3.63	-25.81 ±4.07	-47.17 ±13.53	-45.94 ±14.85	-47.17 ±13.53	-45.94 ±14.85	-47.17 ±13.53	-45.94 ±14.85
Methylparaben	-32.66 ±12.17	-38.37 ±14.82	-33.61 ±11.85	-27.40 ±9.90	-45.65 ±15.58	-26.95 ±10.08	-36.47 ±12.56	-31.43 ±6.34	-23.96 ±1.37	-14.82 ±1.60
Emulsion base	-50.13 ±13.45	-46.87 ±14.99	-50.13 ±13.45	-46.87 ±14.99	-50.13 ±13.45	-46.87 ±14.99	-47.64 ±10.45	-26.28 ±2.17	-47.64 ±10.45	-26.28 ±2.17
Ethylparaben	-19.63 ±1.64	-15.38 ±4.21	-19.71 ±6.32	-14.01 ±4.01	-20.61 ±1.50	-14.99 ±0.91	-16.32 ±1.02	-12.97 ±1.90	-18.39 ±3.62	-14.22 ±2.12
Emulsion base	-25.23 ±2.64	-34.71 ±5.94	-25.23 ±2.64	-34.71 ±5.94	-25.23 ±2.64	-34.71 ±5.94	-25.23 ±2.64	-34.71 ±5.94	-22.09 ±3.63	-25.81 ±4.07
Propylparaben	-24.05 ±2.12	-20.01 ±3.51	-40.20 ±11.55	-16.70 ±2.81	-20.72 ±1.40	-15.63 ±2.60	-37.08 ±13.78	-14.52 ±1.59	-18.30 ±1.45	-14.07 ±2.09

Table 18B The change in pH of paraben submicron emulsions containing Arlasolve DMI during storage for 7 days at ambient temperature.

Formulation	pH±SD									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Emulsion base	5.19 ±0.03	5.13 ±0.03	5.19 ±0.03	5.13 ±0.03	5.23 ±0.04	5.05 ±0.06	5.23 ±0.04	5.05 ±0.06	5.23 ±0.04	5.05 ±0.06
Methylparaben	3.51 ±0.02	3.51 ±0.04	3.52 ±0.01	3.48 ±0.02	3.52 ±0.01	3.50 ±0.006	3.52 ±0.01	3.49 ±0.01	3.53 ±0.006	3.52 ±0.02
Emulsion base	5.82 ±0.05	5.73 ±0.006	5.82 ±0.05	5.73 ±0.006	5.82 ±0.05	5.73 ±0.006	5.94 ±0.01	5.88 ±0.006	5.94 ±0.01	5.88 ±0.006
Ethylparaben	3.53 ±0.006	3.00 ±0.02	3.53 ±0.01	3.01 ±0.02	3.54 ±0.02	3.10 ±0.02	3.52 ±0.03	3.08 ±0.02	3.53 ±0.01	3.11 ±0.02
Emulsion base	5.12 ±0.04	5.05 ±0.06	5.12 ±0.04	5.05 ±0.06	5.12 ±0.04	5.05 ±0.06	5.12 ±0.04	5.05 ±0.06	5.19 ±0.03	5.13 ±0.03
Propylparaben	3.57 ±0.02	3.56 ±0.02	3.55 ±0.006	3.51 ±0.006	3.56 ±0.01	3.50 ±0.01	3.58 ±0.006	3.52 ±0.03	3.58 ±0.006	3.60 ±0.01

Table 19B %Recovery content of alkyl-4-hydroxybenzoate from various concentration of submicron emulsions prepared by extemporaneous addition.

Concentration (%)	Methylparaben			Ethylparaben			Propylparaben		
	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD
30	79.05	80.73	79.89±1.18	94.60	98.70	96.65±2.90	98.02	98.36	98.19±0.24
40	79.23	78.05	78.64±0.83	94.36	94.78	94.56±0.30	101.92	98.52	100.22±2.40
50	85.24	83.54	84.39±1.20	100.53	96.84	98.68±2.61	101.07	103.67	102.37±1.84
60	85.61	85.79	85.70±0.12	103.25	99.72	101.48±2.50	102.97	102.54	102.75±0.31
70	83.28	80.37	81.82±2.05	95.97	98.04	97.00±1.46	100.68	102.51	101.59±1.29

Table 20B % Weight loss of methylparaben submicron emulsions containing Arlasolve DMI after separating by ultracentrifugation.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.4415	0.1101	5.0182	0.2759	5.8457	5.9916	0.1459	2.44	2.48±0.08
	2	0.4472	0.1058	5.0674	0.2172	5.8376	5.9916	0.1540	2.57	
	3	0.5621	0.1101	4.9031	0.2720	5.8473	5.9922	0.1449	2.42	
40%	1	0.4608	0.0734	4.9630	0.3077	5.8049	5.9924	0.1875	3.13	3.41±0.31
	2	0.4268	0.0861	5.0023	0.2753	5.7905	5.9916	0.2011	3.36	
	3	0.4678	0.0570	4.9657	0.2762	5.7667	5.9913	0.2246	3.75	
50%	1	0.4408	0.0819	5.0158	0.2736	5.8121	5.9922	0.1801	3.00	2.87±1.07
	2	0.4760	0.1084	4.9135	0.3270	5.8249	5.9283	0.1034	1.74	
	3	0.4197	0.0909	4.8701	0.3191	5.6998	5.9284	0.2286	3.86	
60%	1	0.4678	0.0626	4.9279	0.3398	5.7981	5.9284	0.1303	2.20	2.58±0.63
	2	0.4721	0.0792	4.8688	0.3123	5.7324	5.9284	0.1960	3.31	
	3	0.4719	0.0631	5.0079	0.3149	5.8578	5.9922	0.1344	2.24	
70%	1	-	-	-	-	-	-	-	-	3.54±0.46
	2	0.5573	0.0263	4.7741	0.3412	5.6989	5.9282	0.2293	3.87	
	3	0.5407	0.0265	4.8195	0.3508	5.7375	5.9282	0.1907	3.22	

Table 21B %Weight loss of ethylparaben submicron emulsions containing Arlasolve DMI after separating by ultracentrifugation.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.4596	0.082	4.4927	0.3621	5.3964	5.5282	0.1318	2.38	2.75±0.36
	2	0.4566	0.1461	4.3564	0.3978	5.3569	5.5287	0.1718	3.11	
	3	0.3710	0.0778	4.5652	0.3623	5.3763	5.5289	0.1526	2.76	
40%	1	0.4395	0.0831	4.0820	0.4210	5.0256	5.5290	0.5034	9.10	4.86±3.79
	2	0.3798	0.1152	4.5002	0.4341	5.4293	5.5286	0.0993	1.80	
	3	0.3878	0.1346	4.3912	0.4115	5.3251	5.5279	0.2028	3.67	
50%	1	0.5401	0.0337	4.3544	0.4296	5.3578	5.5293	0.1715	3.10	2.68±0.40
	2	0.4978	0.0338	4.3988	0.4523	5.3827	5.5287	0.1460	2.64	
	3	0.5583	0.0332	4.4275	0.4182	5.4372	5.5660	0.1288	2.31	
60%	1	0.3840	0.0766	4.5046	0.3761	5.3413	5.5663	0.2250	4.04	4.62±0.81
	2	0.4862	0.1123	4.3446	0.3344	5.2775	5.5665	0.2890	5.19	
	3	-	-	-	-	-	-	-	-	
70%	1	0.4137	0.1124	4.5403	0.3403	5.4067	5.5658	0.1591	2.86	2.91±0.19
	2	0.4772	0.0869	4.5140	0.3355	5.4136	5.5662	0.1526	2.74	
	3	0.3711	0.0772	4.5980	0.3460	5.3923	5.5662	0.1739	3.12	

Table 22B % Weight loss of propylparaben submicron emulsions containing Arlasolve DMI after separating by ultracentrifugation.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.5139	0.1358	4.7698	0.3377	5.7572	5.9473	0.1901	3.20	2.37±0.88
	2	0.4523	0.1555	4.8429	0.4102	5.8609	5.9474	0.0865	1.45	
	3	0.5322	0.1210	4.7841	0.3634	5.8007	5.9479	0.1472	2.47	
40%	1	0.4664	0.0405	4.9000	0.3915	5.7984	5.9476	0.1492	2.51	2.48±0.43
	2	0.4914	0.0844	4.7999	0.3995	5.7752	5.9480	0.1728	2.90	
	3	0.6072	0.1011	4.7380	0.3805	5.8268	5.9484	0.1216	2.04	
50%	1	0.7174	0.1826	4.6004	0.2592	5.7596	5.9474	0.1878	3.16	2.60±0.48
	2	0.7313	0.1912	4.6379	0.2475	5.8079	5.9471	0.1392	2.34	
	3	0.7060	0.1911	4.6724	0.2812	5.8507	5.9887	0.1380	2.30	
60%	1	0.7998	0.2742	4.6436	0.2099	5.9275	5.9894	0.0619	1.03	1.23±0.40
	2	0.7387	0.2519	4.6949	0.2017	5.8872	5.9886	0.1014	1.69	
	3	0.5913	0.2653	4.8907	0.1835	5.9308	5.9884	0.0576	0.96	
70%	1	0.7733	0.2254	4.3353	0.2123	5.5463	5.9891	0.4428	7.39	4.05±2.90
	2	0.7257	0.2283	4.7014	0.2066	5.8620	5.9888	0.1268	2.12	
	3	0.5150	0.2479	4.8492	0.2182	5.8303	5.9884	0.1581	2.64	

Table 23B The analytical amount and recovery of methylparaben from submicron emulsion containing Arlasolve DMI after ultracentrifugation.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0.2748	0.3068	0.7779	0.6849	2.0444	5.9916	2.4951	81.93	82.37 ±0.59
	2	0.3004	0.3464	0.8576	0.5676	2.0720	5.9916	2.4951	83.04	
	3	0.2921	0.2935	0.7810	0.6831	2.0498	5.9922	2.4954	82.14	
40%	1	0.3605	0.2849	1.1430	0.9954	2.7838	5.9924	3.3117	84.06	84.05 ±0.04
	2	0.3751	0.3262	1.1273	0.9557	2.7843	5.9916	3.3112	84.08	
	3	0.4183	0.2430	1.1757	0.9443	2.7813	5.9913	3.3111	84.00	
50%	1	0.5200	0.2625	1.4887	1.1975	3.4687	5.9922	4.0937	84.73	85.23 ±0.91
	2	0.5426	0.3904	1.2144	1.3471	3.4946	5.9283	4.0500	86.28	
	3	0.4961	03015	1.2897	1.3424	3.4296	5.9284	4.0501	84.68	
60%	1	0.7135	0.2380	1.5766	1.6466	4.1747	5.9284	4.8955	85.28	83.01 ±2.23
	2	0.6754	0.2509	1.6378	1.4966	4.0607	5.9284	4.8955	82.95	
	3	0.6362	0.2263	1.6463	1.4900	3.9989	5.9922	4.9482	80.81	
70%	1	-	-	-	-	-	-	-	-	80.56 ±1.78
	2	0.7911	0.1268	1.7128	1.8664	4.4971	5.9282	5.6706	79.30	
	3	0.8700	0.0875	1.7784	1.9038	4.6397	5.9282	5.6706	81.82	

Table 24B The analytical amount and recovery of ethylparaben from submicron emulsion containing Arlasolve DMI after ultracentrifugation.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0.5943	0.2613	0.6364	1.5913	3.0832	5.5282	3.1095	99.15	98.91 ±0.22
	2	0.5211	0.3837	0.6103	1.5586	3.0737	5.5287	3.1098	98.84	
	3	0.5588	0.2086	0.7380	1.5653	3.0707	5.5289	3.1099	98.74	
40%	1	0.9102	0.2335	0.6985	2.1872	4.0295	5.5290	4.2193	95.50	97.53 ±1.76
	2	0.8465	0.3494	0.7651	2.2047	4.1658	5.5286	4.2190	98.74	
	3	0.8441	0.4240	0.6949	2.1855	4.1486	5.5279	4.2184	98.34	
50%	1	1.4769	0.1169	0.8679	2.7319	5.1936	5.5293	5.2514	98.90	98.48 ±0.95
	2	1.4022	0.1173	0.8956	2.7900	5.2051	5.5287	5.2499	99.14	
	3	1.3762	0.1241	0.9005	2.7478	5.1486	5.5660	5.2862	97.40	
60%	1	1.3949	0.4707	1.4488	2.9359	6.2503	5.5663	6.2169	100.54	99.66 ±1.23
	2	1.7048	0.2808	1.4252	2.7312	6.1421	5.5665	6.2171	98.79	
	3	-	-	-	-	-	-	-	-	
70%	1	1.6185	0.9317	1.5826	2.9153	7.0481	5.5658	7.2349	97.42	97.41 ±0.16
	2	1.8337	0.7261	1.6740	2.8256	7.0594	5.5662	7.2354	97.57	
	3	1.5330	0.6985	1.8755	2.9287	7.0357	5.5662	7.2354	97.24	

Table 25B The analytical amount and recovery of propylparaben from submicron emulsion containing Arlasolve DMI after ultracentrifugation.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	1.0413	0.9190	0.5046	2.0431	4.5081	5.9473	4.9056	91.90	95.34 ±4.40
	2	1.2517	0.9839	0.5854	2.0992	4.9202	5.9474	4.9057	100.30	
	3	1.1224	0.8132	0.6524	2.0154	4.6034	5.9479	4.9061	93.83	
40%	1	1.8174	0.6673	1.3263	2.9864	6.7975	5.9476	6.5393	103.95	100.64 ±2.88
	2	1.9262	0.6358	0.8863	3.0415	6.4898	5.9480	6.5398	99.24	
	3	1.8750	0.6662	1.0152	2.9010	6.4573	5.9484	6.5402	98.73	
50%	1	1.8797	2.2696	0.7963	2.3398	7.2855	5.9474	8.2043	88.80	92.71 ±4.62
	2	2.0511	2.2412	0.9245	2.2914	7.5082	5.9471	8.2039	91.52	
	3	2.4110	2.4693	0.6945	2.5061	8.0809	5.9887	8.2613	97.82	
60%	1	2.6252	3.5925	0.7882	1.9830	8.9888	5.9894	9.8702	91.07	91.77 ±1.69
	2	3.0890	3.4563	0.7708	1.9305	9.2466	5.9886	9.8688	93.70	
	3	2.4552	3.6539	0.9554	1.8702	8.9347	5.9884	9.8685	90.54	
70%	1	3.3042	3.4831	1.0252	2.4886	10.3011	5.9891	11.4911	89.64	90.66 ±4.33
	2	2.8312	3.4302	1.3337	2.3944	9.9894	5.9888	11.4906	86.94	
	3	3.5810	3.6795	1.7322	1.9700	10.9628	5.9884	11.4898	95.41	

Table 26B Percentage of drug distribution into various phases of parabens containing Arlasolve DMI submicron emulsions

%drug satutared	%drug distribution (Mean±SD)															
	Oil phase				PC rich phase				Aqueous phase				Mesophase			
	MP	EP	PP	BP	MP	EP	PP	BP	MP	EP	PP	BP	MP	EP	PP	BP
30%	14.06 ±0.55	18.14 ±1.16	24.31 ±1.17	-	15.35 ±1.23	9.25 ±2.92	19.35 ±1.47	-	39.18 ±1.91	21.51 ±2.22	12.42 ±1.56	-	31.41 ±3.48	51.10 ±0.46	43.92 ±1.33	-
40%	13.82 ±1.09	21.08 ±1.30	28.48 ±1.55	-	10.23 ±1.49	8.14 ±2.22	9.98± 0.29	-	41.27 ±0.91	17.48 ±0.82	16.30 ±2.97	-	34.68 ±0.95	53.30 ±0.86	45.24 ±1.49	-
50%	15.00 ±0.53	27.37 ±0.93	27.65 ±2.04	-	9.18 ±1.83	2.30 ±0.09	30.52 ±0.65	-	38.42 ±4.14	17.14 ±0.39	10.61 ±1.88	-	37.40 ±2.51	53.19 ±0.52	31.22 ±0.82	-
60%	16.54 ±0.60	25.04 ±3.84	30.03 ±3.05	-	5.85 ±0.29	6.05 ±2.09	39.41 ±1.82	-	39.76 ±1.77	23.19 ±0.02	9.27 ±1.26	-	37.85 ±1.39	45.72 ±1.77	21.29 ±0.67	-
70%	18.17 ±0.82	23.58 ±2.16	31.03 ±2.34	-	2.35 ±0.66	11.14 ±1.81	33.90 ±0.40	-	38.21 ±0.17	24.28 ±2.16	13.03 ±2.94	-	41.27 ±0.33	41.00 ±0.86	22.03 ±3.52	-

APPENDIX: PART II ALKYL-4-HYDROXYBENZOATE

SHAKING METHOD

Table 27B The change in size of alkyl-4-hydroxybenzoate submicron emulsions (nm) prepared by shaking during storage for 7 days at ambient temperature measured by using Photon Correlation Spectroscopy (PCS).

Formulation	Effective diameter (nm)(polydispersity)	
	t=0	t=7
Emulsion base	240.7(0.048)	244.2(0.087)
Methylparaben	310.2(0.071)	359.6(0.104)
Emulsion base	240.7(0.048)	244.2(0.087)
Propylparaben	365.3(0.112)	393.0(0.069)
Emulsion base	230.8(0.127)	235.8(0.045)
Butylparaben	335.7(0.097)	378.1(0.081)

Table 28B The change in size of alkyl-4-hydroxybenzoate submicron emulsions (nm) prepared by shaking during storage for 7 days at ambient temperature measured by using laser light diffraction (Mastersizer 2000).

Formulation	D[4,3] mean (nm)(span)	
	t=0	t=7
Emulsion base	209(1.645)	224(1.949)
Methylparaben	296(1.728)	391(1.316)
Emulsion base	209(1.645)	224(1.949)
Propylparaben	471(1.552)	478(1.526)
Emulsion base	211(1.706)	222(1.948)
Butylparaben	364(1.555)	428(1.425)

Table 29B The change in zeta potential of alkyl-4-hydroxybenzoate submicron emulsions prepared by shaking during storage for 7 days at ambient temperature measured by Zetaplus™.

Formulation	Zeta Potential±SD	
	t=0	t=7
Emulsion base	-45.53±8.18	-46.57±4.95
Methylparaben	-15.10±2.01	-17.75±1.64
Emulsion base	-45.53±8.18	-46.57±4.95
Propylparaben	-17.62±1.25	-19.53±2.85
Emulsion base	-40.41±5.95	-46.27±4.41
Butylparaben	-19.61±3.08	-19.15±0.58

Table 30BThe change in pH of alkyl-4-hydroxybenzoate submicron emulsions prepared by shaking during storage for 7 days at ambient temperature.

Formulation	pH±SD	
	t=0	t=7
Emulsion base	5.56±0.03	5.39±0.05
Methylparaben	5.56±0.04	5.34±0.04
Emulsion base	5.56±0.03	5.39±0.05
Propylparaben	5.81±0.04	5.72±0.05
Emulsion base	5.30±0.02	5.11±0.006
Butylparaben	5.75±0.04	5.66±0.06

Table 31B % Weight loss after separating by ultracentrifugation of alkyl-4-hydroxybenzoate incorporated in submicron emulsions by shaking.

drug	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
Methylparaben	1	0.5165	0.0946	4.9593	0.3176	5.8880	5.9333	0.0453	0.76	1.37±0.58
	2	0.4805	0.1083	4.9425	0.3162	5.8475	5.9338	0.0863	1.45	
	3	0.4787	0.1118	4.8984	0.3306	5.8195	5.9328	0.1133	1.91	
Propylparaben	1	0.4611	0.1532	4.9628	0.2399	5.8170	5.9336	0.1166	1.96	1.73±0.20
	2	0.5455	0.2134	4.8764	0.2042	5.8395	5.9343	0.0948	1.60	
	3	0.5790	0.2095	4.8483	0.2003	5.8371	5.9344	0.0973	1.64	
Butylparaben	1	0.6251	-	4.7944	0.2598	5.6793	5.9342	0.2549	4.30	3.44±1.15
	2	0.5759	-	4.9164	0.3154	5.8077	5.9348	0.1271	2.14	
	3	0.6245	-	4.7968	0.2829	5.7042	5.9351	0.2309	3.89	

Table 32B The analytical amount and recovery after ultracentrifugation of alkyl-4-hydroxybenzoate incorporated in submicron emulsion by shaking.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich phase (mg)	Amount in aqueous phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
Methylparaben	1	1.5496	0.9725	2.2055	3.1240	7.8516	5.9333	8.0272	97.81	97.70± 0.23
	2	1.5097	0.9478	2.2302	2.9914	7.6791	5.9338	7.8810	97.44	
	3	1.5050	0.8673	2.2030	2.9824	7.5577	5.9328	7.7238	97.85	
Propylparaben	1	2.8842	2.9434	1.2142	2.6889	9.7307	5.9336	10.0636	96.69	94.98± 1.63
	2	2.2739	2.8264	0.6589	1.8756	7.6348	5.9343	8.0536	94.80	
	3	2.3108	2.5850	0.8336	1.8577	7.5872	5.9344	8.1200	93.44	
Butylparaben	1	6.0994	-	1.6042	7.2004	14.9040	5.9342	19.4605	76.58	80.18± 3.27
	2	5.0551	-	1.4802	6.7696	13.3049	5.9348	16.0351	82.97	
	3	4.6286	-	3.1462	4.6141	12.3889	5.9351	15.2998	80.97	

Table 33B Percentage of drug distribution into various phases of submicron emulsion by shaking.

Drug	% Drug distribution (Mean±SD)			
	Oil phase	PC rich phase	Aqueous phase	Mesophase
Methylparaben	19.77±0.13	12.07±0.51	28.76±0.58	39.40±0.42
Propylparaben	29.96±0.44	33.78±3.39	10.70±1.94	25.56±1.79
Butylparaben	38.76±1.90	-	15.76±8.34	45.48±7.25

Table 34B Effect of incorporated methods on the distribution of methylparaben into various phases of submicron emulsions.

Method of incorporation	% Drug distribution (Mean±SD)			
	Oil phase	PC rich phase	Aqueous phase	Mesophase
De novo emulsification (70% saturated oil solubility)	22.67±6.36	11.39±1.44	36.31±5.48	29.63±3.02
Extemporaneous addition (70% saturated oil solubility)	18.17±0.82	2.35±0.66	38.21±0.17	41.27±0.33
Shaking	19.77±0.13	12.07±0.51	28.76±0.58	39.40±0.42

Table 35B Effect of incorporated methods on the distribution of ethylparaben into various phases of submicron emulsions.

Method of incorporation	% Drug distribution (Mean±SD)			
	Oil phase	PC rich phase	Aqueous phase	Mesophase
De novo emulsification (70% saturated oil solubility)	29.41±4.38	29.79±4.22	21.52±0.85	19.28±1.48
Extemporaneous addition (70% saturated oil solubility)	23.58±2.16	11.14±1.81	24.28±2.16	41.00±0.86
Shaking	-	-	-	-

Table 36B Effect of incorporated methods on the distribution of propylparaben into various phases of submicron emulsions.

Method of incorporation	% Drug distribution (Mean±SD)			
	Oil phase	PC rich phase	Aqueous phase	Mesophase
De novo emulsification (70% saturated oil solubility)	24.74±7.67	15.94±3.09	16.71±6.24	45.49±13.24
Extemporaneous addition (70% saturated oil solubility)	31.03±2.34	33.90±0.40	13.03±2.94	22.03±3.52
Shaking	29.96±0.44	33.78±3.39	10.70±1.94	25.56±1.79

Table 37B Effect of incorporated methods on the distribution of butylparaben into various phases of submicron emulsions.

Method of incorporation	% Drug distribution (Mean±SD)			
	Oil phase	PC rich phase	Aqueous phase	Mesophase
De novo emulsification (70% saturated oil solubility)	35.81±3.51	10.11±0.43	9.32±2.94	44.76±6.35
Extemporaneous addition (70% saturated oil solubility)	-	-	-	-
Shaking	38.76±1.90	-	15.76±8.34	45.48±7.25

APPENDIX: PART III BENZODIAZEPINE DRUGS

DE NOVO EMULSIFICATION

Table 1C The change in size of benzodiazepine submicron emulsions (nm) during storage for 7 days at ambient temperature measured by using Photon Correlation Spectroscopy (PCS).

Formulation	Effective mean diameter(nm)(polydispersity)									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Alprazolam	223.7 (0.059)	225.9 (0.058)	218.5 (0.103)	219.0 (0.075)	222.5 (0.078)	222.1 (0.058)	213.1 (0.094)	219.9 (0.076)	208.3 (0.098)	209.3 (0.081)
Clonazepam	219.0 (0.099)	229.9 (0.060)	218.2 (0.096)	224.2 (0.094)	213.6 (0.074)	218.4 (0.125)	218.6 (0.087)	228.0 (0.088)	221.3 (0.078)	224.9 (0.083)
Diazepam	225.4 (0.081)	233.7 (0.112)	226.9 (0.098)	234.2 (0.053)	233.8 (0.083)	290.2 (0.247)	229.5 (0.12)	226.3 (0.107)	217.8 (0.163)	218.3 (0.115)
Lorazepam	230.2 (0.092)	238.9 (0.08)	229.6 (0.090)	237.0 (0.165)	221.9 (0.08)	229.6 (0.094)	227.7 (0.095)	237.0 (0.113)	224.3 (0.07)	231.7 (0.131)

Table 2C The change in size of benzodiazepine submicron emulsions (nm) during storage for 7 days at ambient temperature measured by using laser light diffraction (Mastersizer 2000).

Formulation	D(4,3)(span)									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Alprazolam	163 (1.503)	165 (1.530)	163 (1.505)	161 (1.508)	153 (1.453)	155 (1.468)	151 (1.436)	153 (1.450)	147 (1.414)	155 (1.446)
Clonazepam	158 (1.498)	178 (1.586)	151 (1.445)	165 (1.537)	149 (1.433)	168 (1.532)	160 (1.486)	174 (1.571)	167 (1.528)	173 (1.563)
Diazepam	182 (1.608)	181 (1.566)	175 (1.578)	194 (1.569)	189 (1.613)	190 (1.585)	181 (1.591)	185 (1.563)	156 (1.474)	171 (1.555)
Lorazepam	172 (1.538)	197 (1.617)	177 (1.580)	175 (1.547)	169 (1.545)	207 (1.625)	171 (1.559)	174 (1.537)	171 (1.550)	186 (1.587)

Table 3C The change in zeta potential of benzodiazepine submicron emulsions during storage for 7 days at ambient temperature measured by Zetaplus™.

Formulation	Zeta potential mean ± SD									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Alprazolam	-25.37 ±8.42	-11.26 ±6.40	-37.78 ±7.84	-27.06 ±6.22	-23.77 ±7.74	-21.73 ±7.42	-20.41 ±7.84	-12.58 ±1.57	-13.54 ±1.36	-16.38 ±1.87
Clonazepam	-15.98 ±3.39	-21.57 ±1.75	-14.41 ±4.90	-17.88 ±3.12	-13.74 ±1.74	-21.62 ±2.96	-18.32 ±2.34	-20.63 ±4.02	-11.58 ±2.20	-16.99 ±1.45
Diazepam	-15.81 ±2.38	-21.51 ±3.70	-14.09 ±1.56	-22.17 ±2.82	-14.37 ±1.65	-23.83 ±3.95	-15.03 ±2.39	-21.76 ±3.04	-17.36 ±0.96	-24.86 ±1.78
Lorazepam	-14.42 ±1.24	-19.86 ±2.46	-15.86 ±2.96	-19.96 ±2.59	-17.22 ±2.28	-23.34 ±4.37	-16.86 ±2.82	-18.41 ±1.77	-17.47 ±2.78	-22.13 ±1.98

Table 4C The change in pH of benzodiazepine submicron emulsions during storage for 7 days at ambient temperature.

Formulation	Mean pH±SD									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Alprazolam	6.04 ±0.03	5.84 ±0.05	6.11 ±0.02	5.69 ±0.03	5.90 ±0.04	5.67 ±0.07	5.82 ±0.05	5.50 ±0.03	5.82 ±0.02	5.35 ±0.09
Clonazepam	5.77 ±0.08	5.66 ±0.07	5.94 ±0.03	5.72 ±0.03	5.98 ±0.03	5.73 ±0.03	6.01 ±0.02	5.73 ±0.01	6.05 ±0.02	5.90 ±0.02
Diazepam	6.10 ±0.03	4.93 ±0.01	6.26 ±0.04	4.92 ±0.02	6.25 ±0.04	5.36 ±0.03	6.47 ±0.06	5.68 ±0.01	5.54 ±0.03	4.44 ±0.02
Lorazepam	6.33 ±0.02	5.90 ±0.01	6.33 ±0.02	6.05 ±0.03	6.36 ±0.03	5.98 ±0.03	6.17 ±0.02	6.01 ±0.06	6.00 ±0.02	5.31 ±0.02

Table 5C %Recovery content of benzodiazepines from various concentration of submicron emulsions prepared by de novo method.

Concentration (%)	Alprazolam			Clonazepam			Diazepam			Lorazepam		
	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD
30	100.50	97.00	98.75±2.48	104.10	102.04	103.07±1.46	107.87	109.63	108.75±1.24	101.02	101.94	101.48±0.65
40	92.26	92.58	92.42±0.22	100.92	102.17	101.54±0.88	104.79	103.58	104.18±0.86	102.16	99.34	100.75±2.00
50	90.01	90.63	90.32±0.44	104.76	106.68	105.72±1.36	96.98	85.46	91.22±8.14	98.87	97.52	98.19±0.95
60	94.56	92.52	93.54±1.44	105.23	106.27	105.75±0.74	103.82	96.96	100.39±4.85	99.90	100.08	99.99±0.12
70	94.79	93.83	94.31±0.68	102.98	101.09	102.04±1.34	104.41	106.49	105.45±1.47	101.03	98.84	99.93±1.55

Table 6C %Weight loss after separating by ultracentrifugation of alprazolam submicron emulsionss prepared by de novo emulsification.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.4858	0.3458	6.2678	0.1539	7.2533	7.3378	0.0845	1.15	0.98±0.15
	2	0.3798	0.3369	6.3511	0.2029	7.2707	7.3384	0.0677	0.92	
	3	0.3544	0.3731	6.3722	0.1735	7.2732	7.3374	0.0642	0.87	
40%	1	0.4123	0.3687	6.3155	0.1821	7.2786	7.3369	0.0583	0.79	0.76±0.07
	2	0.4509	0.4395	6.2191	0.1683	7.2778	7.3381	0.0603	0.82	
	3	0.4529	0.3652	6.3056	0.1639	7.2876	7.3375	0.0499	0.68	
50%	1	0.4041	0.3455	6.3548	0.1517	7.2561	7.3764	0.1203	1.63	1.46±0.19
	2	0.5651	0.3484	6.1956	0.1564	7.2655	7.3771	0.1116	1.51	
	3	0.4124	0.3294	6.3772	0.1654	7.2844	7.3769	0.0925	1.25	
60%	1	0.4282	0.3784	6.2836	0.1830	7.2732	7.3770	0.1038	1.41	1.41±0.20
	2	0.4437	0.3849	6.2548	0.1757	7.2591	7.3775	0.1184	1.60	
	3	0.4103	0.3665	6.3549	0.1560	7.2877	7.3771	0.0894	1.21	
70%	1	0.3617	0.3904	5.9804	0.1794	6.9119	7.1409	0.2290	3.21	2.26±1.37
	2	0.3795	0.3861	5.9303	0.2396	6.9355	7.1408	0.2053	2.88	
	3	0.4223	0.3944	6.1346	0.1403	7.0916	7.1409	0.0493	0.69	

Table 7C %Weight loss after separating by ultracentrifugation of clonazepam submicron emulsionss prepared by de novo emulsification.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.4335	0.2688	6.0386	0.1628	6.9037	7.0053	0.1016	1.45	1.57±0.12
	2	0.4247	0.2686	6.0119	0.1908	6.8960	7.0051	0.1091	1.56	
	3	0.4123	0.2592	6.0584	0.1565	6.8864	7.0053	0.1189	1.70	
40%	1	0.4391	0.3172	5.9647	0.1717	6.8927	7.0049	0.1122	1.60	1.75±0.30
	2	0.4515	0.2931	5.9572	0.1571	6.8589	7.0055	0.1466	2.09	
	3	0.4370	0.3022	5.9948	0.1618	6.8958	7.0051	0.1093	1.56	
50%	1	0.5531	0.2718	5.9295	0.1585	6.9129	7.0055	0.0926	1.32	1.97±1.33
	2	0.4922	0.3026	5.9711	0.1628	6.9287	7.0054	0.0767	1.09	
	3	0.4238	0.2428	5.9234	0.1906	6.7806	7.0269	0.2463	3.50	
60%	1	0.3815	0.2759	5.9636	0.1961	6.8171	7.0266	0.2095	2.98	2.69±1.13
	2	0.4232	0.2705	5.8982	0.1789	6.7708	7.0272	0.2564	3.65	
	3	0.4291	0.2827	6.0604	0.1633	6.9255	7.0273	0.1018	1.45	
70%	1	0.4094	0.2761	6.1186	0.1451	6.9492	7.0270	0.0778	1.11	1.24±0.15
	2	0.4182	0.2852	6.0974	0.1420	6.9428	7.0269	0.0841	1.20	
	3	0.3532	0.3076	6.1131	0.1544	6.9283	7.0267	0.0984	1.40	

Table 8C %Weight loss after separating by ultracentrifugation of diazepam submicron emulsionss prepared by de novo emulsification.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.4739	0.3581	5.8924	0.1770	6.9014	7.0006	0.0992	1.42	1.93±1.09
	2	0.4519	0.3714	5.7566	0.1969	6.7768	7.0000	0.2233	3.19	
	3	0.5133	0.3581	5.8859	0.1598	6.9171	7.0005	0.0834	1.19	
40%	1	0.4171	0.3600	5.9789	0.1704	6.9264	7.0008	0.0744	1.06	0.95±0.10
	2	0.4387	0.3265	6.0074	0.1669	6.9395	7.0000	0.0605	0.86	
	3	0.4254	0.3239	6.0430	0.1416	6.9339	7.0000	0.0661	0.94	
50%	1	0.4454	0.3054	6.0406	0.1724	6.9638	7.0463	0.0825	1.17	1.27±0.43
	2	0.3779	0.3348	6.0817	0.1895	6.9839	7.0466	0.0627	0.89	
	3	0.4323	0.3347	6.0322	0.1247	6.9239	7.0462	0.1223	1.74	
60%	1	0.4619	0.3300	5.8836	0.1222	6.7977	7.0465	0.2488	3.53	1.95±1.37
	2	0.4357	0.3766	5.9774	0.1818	6.9715	7.0467	0.0752	1.07	
	3	0.4612	0.3707	5.9671	0.1597	6.9587	7.0467	0.0880	1.25	
70%	1	0.5976	0.3607	5.7551	0.1761	6.8895	7.0322	0.1427	2.03	2.58±0.49
	2	0.5101	0.3400	5.7302	0.2421	6.8224	7.0319	0.2095	2.98	
	3	0.5175	0.3369	5.7500	0.2346	6.8390	7.0321	0.1931	2.74	

Table 9C %Weight loss after separating by ultracentrifugation of lorazepam submicron emulsionss prepared by de novo emulsification.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.4339	0.3586	6.3329	0.1644	7.2898	7.3745	0.0847	1.15	1.60±1.19
	2	0.4042	0.3211	6.2605	0.1710	7.1568	7.3743	0.2175	2.95	
	3	0.4403	0.3625	6.3925	0.1282	7.3235	7.3746	0.0511	0.69	
40%	1	0.4628	0.3319	6.3054	0.2018	7.3019	7.3746	0.0727	0.98	0.94±0.21
	2	0.3563	0.3237	6.4352	0.2058	7.3210	7.3742	0.0532	0.72	
	3	0.6529	0.1531	5.9146	0.2483	6.9689	7.0488	0.0799	1.13	
50%	1	0.4866	0.2111	5.8890	0.2325	6.8192	7.0489	0.2297	3.26	2.55±1.40
	2	0.5909	0.1750	5.8599	0.1789	6.8047	7.0482	0.2435	3.45	
	3	0.5592	0.1759	6.0621	0.1853	6.9825	7.0481	0.0656	0.93	
60%	1	0.7201	0.2783	5.7952	0.1894	6.9830	7.0488	0.0658	0.93	1.26±0.58
	2	0.4704	0.2667	6.0646	0.1830	6.9847	7.0492	0.0645	0.91	
	3	0.4782	0.2762	5.9264	0.2316	6.9124	7.0487	0.1363	1.93	
70%	1	0.2670	0.2921	6.4265	0.1916	7.1772	7.3743	0.1971	2.67	1.30±1.21
	2	0.3692	0.3394	6.3801	0.2233	7.3120	7.3741	0.0621	0.84	
	3	0.4042	0.3707	6.4041	0.1672	7.3462	7.3748	0.0286	0.39	

Table 10C The analytical amount and recovery after ultracentrifugation of alprazolam submicron emulsion prepared by de novo emulsification.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0.0114	0.0335	0.0745	0.0197	0.1391	7.3378	0.1602	86.78	88.66 ±5.11
	2	0.0071	0.0314	0.0891	0.0236	0.1512	7.3384	0.1602	94.44	
	3	0.0089	0.0347	0.0735	0.0186	0.1357	7.3374	0.1602	84.75	
40%	1	0.0091	0.0434	0.0908	0.0270	0.1703	7.3369	0.1974	86.30	87.67 ±2.21
	2	0.0092	0.0522	0.0836	0.0257	0.1707	7.3381	0.1974	86.48	
	3	0.0087	0.0558	0.0892	0.0243	0.1781	7.3375	0.1974	90.21	
50%	1	0.0092	0.0880	0.0982	0.0511	0.2466	7.3764	0.2545	96.91	95.55 ±1.18
	2	0.0088	0.0920	0.0932	0.0475	0.2415	7.3771	0.2545	94.89	
	3	0.0099	0.0839	0.1016	0.0459	0.2414	7.3769	0.2545	94.85	
60%	1	0.0111	0.1166	0.1235	0.0492	0.3004	7.3770	0.3128	96.04	95.83 ±1.42
	2	0.0108	0.1068	0.1242	0.0532	0.2950	7.3775	0.3128	94.32	
	3	0.0122	0.1145	0.1292	0.0479	0.3038	7.3771	0.3128	97.14	
70%	1	0.0110	0.1309	0.1242	0.0535	0.3196	7.1409	0.3490	91.59	93.94 ±2.12
	2	0.0131	0.1176	0.1335	0.0696	0.3339	7.1408	0.3490	95.68	
	3	0.0137	0.1270	0.1309	0.0585	0.3300	7.1409	0.3490	94.57	

Table 11C The analytical amount and recovery after ultracentrifugation of clonazepam submicron emulsion prepared by de novo emulsification.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0.0082	0.0464	0.0226	0.0144	0.0917	7.0053	0.0917	100.00	93.20 ±9.62
	2	0.0051	0.0438	0.0205	0.0098	0.0792	7.0051	0.0917	86.40	
	3	0.0060	0.0502	0.0205	0.0509	0.1275	7.0053	0.0917	139.08	
40%	1	0.0021	0.0638	0.0309	0.0126	0.1094	7.0049	0.1095	99.96	98.79 ±5.78
	2	0.0058	0.0699	0.0318	0.0063	0.1138	7.0055	0.1095	103.90	
	3	0.0048	0.0559	0.0318	0.0087	0.1013	7.0051	0.1095	92.52	
50%	1	0.0063	0.0560	0.0419	0.0121	0.1162	7.0055	0.1376	84.47	90.16 ±5.32
	2	0.0070	0.0625	0.0403	0.0154	0.1252	7.0054	0.1376	90.97	
	3	0.0052	0.0693	0.0332	0.0234	0.1312	7.0269	0.1380	95.03	
60%	1	0.0038	0.0721	0.0406	0.0200	0.1365	7.0266	0.1730	78.93	85.28 ±8.42
	2	0.0061	0.0809	0.0424	0.0347	0.1640	7.0272	0.1730	94.83	
	3	0.0046	0.0732	0.0403	0.0239	0.1420	7.0273	0.1730	82.08	
70%	1	0.0038	0.0611	0.0563	0.0533	0.1746	7.0270	0.1891	92.30	83.06 ±8.45
	2	0.0047	0.0797	0.0481	0.0210	0.1535	7.0269	0.1891	81.17	
	3	0.0058	0.0847	0.0405	0.0122	0.1432	7.0267	0.1891	75.71	

Table 12C The analytical amount and recovery after ultracentrifugation of diazepam submicron emulsion prepared by de novo emulsification.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq.phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	1.0851	1.0750	0.2736	0.3214	2.7552	7.0006	2.9102	94.67	91.72 ±3.03
	2	1.0023	0.9406	0.2751	0.3607	2.5788	7.0000	2.9100	88.62	
	3	1.1037	1.0002	0.2597	0.3101	2.6737	7.0005	2.9102	91.87	
40%	1	1.3894	1.4930	0.3274	0.4468	3.6567	7.0008	3.8904	93.99	89.00 ±4.32
	2	1.2918	1.2260	0.3941	0.4494	3.3612	7.0000	3.8900	86.41	
	3	1.2023	1.2597	0.4732	0.4334	3.3686	7.0000	3.8900	86.60	
50%	1	1.9252	1.5172	0.6855	0.6437	4.7717	7.0463	4.9123	97.14	92.82 ±3.93
	2	1.3337	1.6282	0.7494	0.8028	4.5141	7.0466	4.9125	91.89	
	3	1.6701	1.5851	0.6724	0.4658	4.3933	7.0462	4.9122	89.44	
60%	1	2.0593	1.8141	0.6374	0.4286	4.9394	7.0465	5.8083	85.04	90.37 ±4.96
	2	1.8680	2.0590	0.8114	0.7704	5.5087	7.0467	5.8085	94.84	
	3	2.0338	1.9365	0.6464	0.6818	5.2986	7.0467	5.8085	91.22	
70%	1	3.1794	1.7938	0.5799	0.5574	6.1104	7.0322	6.7509	90.51	88.39 ±1.85
	2	2.7645	1.6509	0.7732	0.6914	5.8800	7.0319	6.7506	87.10	
	3	3.0658	1.5859	0.7316	0.5283	5.9116	7.0321	6.7508	87.57	

Table 13C The analytical amount and recovery after ultracentrifugation of lorazepam submicron emulsion prepared by de novo emulsification.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0.0099	0.0904	0.0845	0.0621	0.2469	7.3745	0.2597	95.09	90.99 ±3.55
	2	0.0089	0.0788	0.0846	0.0586	0.2309	7.3743	0.2597	88.92	
	3	0.0084	0.0792	0.0852	0.0583	0.2310	7.3746	0.2597	88.96	
40%	1	0.0104	0.0883	0.1145	0.0790	0.2922	7.3746	0.3105	94.12	92.19 ±2.61
	2	0.0089	0.0825	0.1264	0.0717	0.2895	7.3742	0.3104	93.24	
	3	0.0156	0.0805	0.1081	0.0605	0.2648	7.0488	0.2968	89.22	
50%	1	0.0150	0.0873	0.1175	0.1171	0.3369	7.0489	0.3763	89.54	88.55 ±1.24
	2	0.0147	0.0807	0.1250	0.1076	0.3280	7.0482	0.3763	87.16	
	3	0.0155	0.0770	0.1385	0.1037	0.3346	7.0481	0.3763	88.94	
60%	1	0.0143	0.1940	0.1227	0.1285	0.4596	7.0488	0.4605	99.80	96.94 ±3.41
	2	0.0155	0.1817	0.1231	0.1303	0.4506	7.0492	0.4605	97.85	
	3	0.0157	0.1744	0.1312	0.1076	0.4290	7.0487	0.4605	93.17	
70%	1	0.0112	0.1975	0.1763	0.1222	0.5071	7.3743	0.5443	93.17	96.22 ±2.72
	2	0.0137	0.2074	0.1808	0.1267	0.5286	7.3741	0.5443	97.11	
	3	0.0149	0.2039	0.1976	0.1192	0.5355	7.3748	0.5444	98.38	

Table 14C Percentage of drug distribution into various phases of submicron emulsion prepared by de novo emulsification.

%drug satutared	%drug distribution (Mean±SD)															
	Oil phase				PC rich phase				Aqueous phase				Mesophase			
	A	C	D	L	A	C	D	L	A	C	D	L	A	C	D	L
30%	6.49 ±1.74	6.68 ±2.15	39.84 ±1.27	3.84 ±1.20	23.47 ±2.45	48.44 ±8.23	37.63 ±1.28	35.00 ±1.40	55.53 ±2.92	22.21 ±5.37	11.21 ±2.41	35.91 ±1.46	14.51 ±0.99	22.67 ±15.03	12.42 ±1.36	25.25 ±0.11
40%	5.21 ±0.28	3.93 ±1.73	37.37 ±1.47	4.18 ±1.51	29.15 ±3.19	58.29 ±3.13	38.23 ±2.30	29.70 ±1.05	50.78 ±2.25	29.21 ±1.91	11.58 ±2.55	41.23 ±2.26	14.85 ±1.11	8.56 ±3.03	12.82 ±0.56	24.88 ±2.08
50%	3.84 ±0.26	4.99 ±0.91	35.97 ±5.68	4.53 ±0.08	36.18 ±1.72	50.32 ±2.35	34.65 ±2.47	24.51 ±1.44	40.18 ±1.77	31.18 ±5.40	15.42 ±1.12	38.12 ±3.25	19.81 ±0.87	13.51 ±3.90	13.58 ±4.15	32.84 ±1.89
60%	3.79 ±0.20	3.26 ±0.46	37.99 ±3.90	3.41 ±0.28	37.57 ±1.30	51.22 ±1.79	36.88 ±0.44	41.06 ±1.00	41.91 ±0.73	27.97 ±1.98	12.63 ±1.92	28.20 ±2.09	16.73 ±1.17	17.54 ±3.31	11.84 ±2.80	27.33 ±2.00
70%	3.84 ±0.35	3.10 ±0.93	50.30 ±2.85	2.53 ±0.29	38.22 ±2.87	48.70 ±12.40	28.09 ±1.26	38.74 ±0.60	39.50 ±0.57	30.63 ±2.08	11.67 ±1.93	35.29 ±1.42	18.43 ±2.15	17.57 ±11.54	9.94 ±1.58	23.91 ±0.22

A = Alprazolam

C = Clonazepam

D = Diazepam

L = Lorazepam

APPENDIX: PART III BENZODIAZEPINE DRUGS

EXTEMPORANEOUS ADDITION

Table 15C The change in size of benzodiazepine submicron emulsions (nm) containing Arlasolve DMI during storage for 7 days at ambient temperature measured by using Photon Correlation Spectroscopy (PCS).

Formulation	Effective diameter (nm)(polydispersity)									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Emulsion base	270.1 (0.109)	277.2 (0.047)	270.1 (0.109)	277.2 (0.047)	270.1 (0.109)	277.2 (0.047)	263.9 (0.166)	272.4 (0.079)	266.3 (0.095)	278.5 (0.102)
Alprazolam	276.0 (0.065)	275.0 (0.123)	274.0 (0.096)	272.6 (0.112)	270.6 (0.084)	271.6 (0.102)	274.8 (0.117)	277.8 (0.104)	269.6 (0.074)	284.9 (0.005)
Emulsion base	283.3 (0.081)	296.0 (0.115)	283.3 (0.081)	296.0 (0.115)	283.3 (0.081)	296.0 (0.115)	263.9 (0.166)	272.4 (0.079)	255.0 (0.096)	264.3 (0.165)
Clonazepam	295.8 (0.054)	288.2 (0.074)	305.6 (0.145)	292.6 (0.098)	292.2 (0.051)	296.9 (0.114)	268.2 (0.105)	277.3 (0.077)	256.6 (0.157)	268.1 (0.113)
Emulsion base	268.4 (0.052)	258.3 (0.098)	268.4 (0.052)	258.3 (0.098)	268.4 (0.052)	258.3 (0.098)	268.4 (0.052)	258.3 (0.098)	263.9 (0.166)	272.4 (0.079)
Diazepam	272.0 (0.100)	264.2 (0.123)	273.9 (0.086)	267.1 (0.067)	276.2 (0.106)	264.5 (0.139)	275.2 (0.116)	255.3 (0.173)	259.9 (0.123)	254.4 (0.203)
Emulsion base	355.4 (0.167)	333.9 (1.900)	263.9 (0.166)	272.4 (0.079)	355.4 (0.167)	333.9 (1.900)	355.4 (0.167)	333.9 (1.900)	244.7 (0.153)	269.2 (0.124)
Lorazepam	375.0 (0.125)	347.0 (0.176)	276.9 (0.115)	273.9 (0.129)	363.5 (0.219)	328.5 (0.212)	359.8 (0.166)	371.1 (0.185)	265.1 (0.048)	274.6 (0.121)

Table 16C The change in size of benzodiazepine submicron emulsions (nm) containing Arlasolve DMI during storage for 7 days at ambient temperature measured by using laser light diffraction (Mastersizer 2000).

Formulation	D[4,3] mean (nm) (span)									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Emulsion base	259 (1.441)	264 (1.404)	259 (1.441)	264 (1.404)	259 (1.441)	264 (1.404)	287 (1.512)	295 (1.465)	331 (1.822)	340 (1.871)
Alprazolam	275 (1.502)	276 (1.493)	262 (1.423)	282 (1.456)	276 (1.491)	269 (1.473)	301 (1.646)	299 (1.616)	309 (1.525)	321 (1.559)
Emulsion base	349 (1.359)	356 (1.293)	349 (1.359)	356 (1.239)	349 (1.359)	356 (1.239)	287 (1.512)	295 (1.465)	293 (1.456)	299 (1.501)
Clonazepam	334 (1.670)	364 (1.486)	339 (1.479)	345 (1.415)	358 (1.530)	353 (1.564)	294 (1.584)	301 (1.549)	310 (1.571)	322 (1.599)
Emulsion base	270 (1.550)	245 (1.618)	270 (1.550)	245 (1.618)	270 (1.550)	245 (1.618)	270 (1.550)	245 (1.618)	274 (1.656)	260 (1.697)
Diazepam	291 (1.596)	281 (1.662)	282 (1.549)	277 (1.580)	284 (1.518)	277 (1.572)	305 (1.667)	300 (1.689)	283 (1.639)	279 (1.612)
Emulsion base	522 (1.807)	506 (1.893)	287 (1.512)	295 (1.465)	522 (1.807)	506 (1.893)	522 (1.807)	506 (1.893)	279 (1.693)	265 (1.689)
Lorazepam	573 (2.218)	519 (1.890)	320 (1.560)	304 (1.692)	512 (2.255)	477 (1.648)	529 (1.838)	485 (2.083)	305 (1.819)	290 (1.901)

Table 17CThe change in zeta potential of benzodiazepine submicron emulsions containing Arlasolve DMI during storage for 7 days at ambient temperature measured by Zetaplus™.

Formulation	Zeta Potential±SD									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Emulsion base	-24.84 ±2.71	-25.94 ±1.69	-24.84 ±2.71	-25.94 ±1.69	-24.84 ±2.71	-25.94 ±1.69	-23.83 ±4.79	-43.66 ±1.84	-22.49 ±2.07	-26.14 ±1.76
Alprazolam	-17.09 ±1.54	-18.66 ±2.27	-18.59 ±1.78	-19.90 ±2.94	-17.93 ±0.93	-17.89 ±2.03	-25.84 ±6.18	-18.34 ±3.19	-25.37 ±3.80	-26.65 ±1.56
Emulsion base	-33.14 ±2.73	-36.86 ±1.66	-33.14 ±2.73	-36.86 ±1.66	-33.14 ±2.73	-36.86 ±1.66	-23.83 ±4.79	-43.66 ±1.84	-19.02 ±1.64	-30.48 ±1.42
Clonazepam	-18.28 ±3.68	-26.93 ±5.83	-18.02 ±4.25	-20.18 ±6.29	-18.70 ±2.54	-33.47 ±4.20	-20.27 ±2.67	-18.23 ±1.82	-18.61 ±0.89	-25.25 ±1.62
Emulsion base	-14.33 ±1.86	-14.93 ±1.21	-14.33 ±1.86	-14.93 ±1.21	-14.33 ±1.86	-14.93 ±1.21	-14.33 ±1.86	-14.93 ±1.21	-16.29 ±2.05	-14.91 ±1.73
Diazepam	-12.79 ±2.27	-14.90 ±4.04	-11.02 ±2.87	-15.22 ±1.19	-13.26 ±1.84	-21.43 ±4.34	-17.02 ±3.72	-18.93 ±2.61	-20.91 ±3.84	-27.22 ±4.60
Emulsion base	-25.56 ±1.78	-54.29 ±2.10	-23.83 ±4.79	-43.66 ±1.84	-25.56 ±1.78	-54.29 ±2.10	-25.56 ±1.78	-54.29 ±2.10	-13.53 ±1.34	-16.14 ±1.74
Lorazepam	-7.58 ±1.19	-22.05 ±2.21	-14.45 ±1.81	-26.24 ±1.46	-18.30 ±0.73	-15.58 ±1.78	-24.51 ±2.15	-25.57 ±1.32	-18.12 ±2.33	-20.77 ±1.37

Table 18C The change in pH of benzodiazepine submicron emulsions containing Arlasolve DMI during storage for 7 days at ambient temperature.

Formulation	pH±SD									
	30%		40%		50%		60%		70%	
	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7	t=0	t=7
Emulsion base	5.47 ±0.03	5.58 ±0.10	5.47 ±0.03	5.58 ±0.10	5.47 ±0.03	5.58 ±0.10	5.43 ±0.03	5.76 ±0.08	5.52 ±0.01	5.45 ±0.02
Alprazolam	3.64 ±0.04	3.59 ±0.02	3.61 ±0.01	3.52 ±0.02	3.63 ±0.006	3.56 ±0.02	3.66 ±0.01	3.49 ±0.02	3.53 ±0.02	3.47 ±0.05
Emulsion base	5.38 ±0.01	5.30 ±0.02	5.38 ±0.01	5.30 ±0.02	5.38 ±0.01	5.30 ±0.02	5.43 ±0.03	5.76 ±0.08	5.34 ±0.02	5.48 ±0.01
Clonazepam	3.41 ±0.03	3.42 ±0.02	3.62 ±0.01	3.49 ±0.02	3.58 ±0.02	3.53 ±0.04	3.55 ±0.01	3.46 ±0.02	3.52 ±0.01	3.44 ±0.02
Emulsion base	5.32 ±0.02	4.66 ±0.02	5.32 ±0.02	4.66 ±0.02	5.32 ±0.02	4.66 ±0.02	5.32 ±0.02	4.66 ±0.02	5.74 ±0.01	5.01 ±0.05
Diazepam	3.53 ±0.03	3.49 ±0.04	3.91 ±0.02	3.50 ±0.006	3.57 ±0.01	3.49 ±0.01	3.58 ±0.02	3.52 ±0.006	3.64 ±0.02	3.56 ±0.03
Emulsion base	5.03 ±0.03	4.89 ±0.01	5.43 ±0.03	5.79 ±0.08	5.03 ±0.03	4.89 ±0.01	5.03 ±0.03	4.89 ±0.01	5.72 ±0.006	4.93 ±0.006
Lorazepam	3.59 ±0.01	3.48 ±0.03	3.56 ±0.01	3.55 ±0.08	3.61 ±0.02	3.62 ±0.04	3.59 ±0.01	3.66 ±0.05	3.48 ±0.01	3.39 ±0.01

Table 19C %Recovery content of benzodiazepines from various concentration of submicron emulsions prepared by extemporaneous addition.

Concentration (%)	Alprazolam			Clonazepam			Diazepam			Lorazepam		
	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD	1	2	Mean±SD
30	91.80	83.97	87.89±5.54	90.28	95.96	93.12±4.02	101.11	96.66	98.89±3.15	85.79	87.99	86.89±1.55
40	104.47	107.69	106.08±2.27	91.62	92.61	92.12±0.70	99.44	100.55	99.99±0.78	99.93	86.21	93.07±9.70
50	95.44	86.26	90.85±6.49	93.73	94.46	94.10±0.52	98.10	100.58	99.34±1.75	103.06	98.28	100.67±3.38
60	87.12	83.80	85.46±2.34	96.19	94.45	95.32±1.23	96.97	101.13	99.05±2.94	89.90	91.18	90.54±0.90
70	84.13	83.90	84.02±0.16	95.06	88.79	91.92±4.43	90.94	94.54	92.74±2.54	92.33	93.30	92.82±0.68

Table 20C %Weight loss of alprazolam submicron emulsions containing Arlasolve DMI after separating by ultracentrifugation.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.4999	0.2204	4.9589	0.2331	5.9123	6.1343	0.2220	3.62	2.74±1.26
	2	0.4293	0.2224	5.0999	0.1798	5.9314	6.1340	0.2026	3.30	
	3	0.4379	0.2315	5.2243	0.1609	6.0546	6.1340	0.0794	1.29	
40%	1	0.3426	0.2444	5.1985	0.2020	5.9875	6.1345	0.1470	2.40	2.13±0.64
	2	0.3379	0.2371	5.2043	0.1973	5.9766	6.1350	0.1584	2.58	
	3	0.3363	0.2741	5.2326	0.2061	6.0491	6.1348	0.0857	1.40	
50%	1	0.4432	0.2093	4.8914	0.2091	5.7530	5.9066	0.1536	2.60	3.11±0.59
	2	0.4268	0.1881	4.9613	0.1546	5.7308	5.9062	0.1754	2.97	
	3	0.5391	0.1855	4.7788	0.1812	5.6846	5.9070	0.2224	3.76	
60%	1	0.4835	0.1765	5.0083	0.1633	5.8316	5.9072	0.0756	1.28	2.06±0.67
	2	0.5091	0.1848	4.9203	0.1465	5.7607	5.9072	0.1465	2.48	
	3	0.4988	0.1776	4.9162	0.1724	5.7650	5.9073	0.1423	2.41	
70%	1	0.4084	0.1540	5.0959	0.1494	5.8077	5.9093	0.1016	1.72	1.61±0.16
	2	0.5207	0.1737	4.9460	0.1816	5.8220	5.9108	0.0888	1.50	
	3	-	-	-	-	-	-	-	-	

Table 21C %Weight loss of clonazepam submicron emulsions containing Arlasolve DMI after separating by ultracentrifugation.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.4054	0.1553	5.0995	0.1890	5.8492	5.9764	0.1272	2.13	2.30±0.14
	2	0.3769	0.1648	5.0886	0.2029	5.8332	5.9761	0.1429	2.39	
	3	0.5185	0.1423	5.0109	0.1627	5.8344	5.9761	0.1417	2.37	
40%	1	0.3892	0.1576	5.0850	0.1760	5.8078	5.9767	0.1689	2.82	2.15±0.85
	2	0.4357	0.1578	5.0886	0.2233	5.9054	5.9765	0.0711	1.19	
	3	0.3943	0.0940	5.1522	0.1900	5.8305	5.9766	0.1461	2.44	
50%	1	0.3100	0.2138	5.1439	0.1208	5.7885	5.9787	0.1902	3.18	2.11±1.10
	2	0.3730	0.1825	5.1893	0.1743	5.9191	5.9780	0.0589	0.98	
	3	0.3905	0.1828	5.1167	0.1579	5.8479	5.9783	0.1304	2.18	
60%	1	0.3999	0.1663	5.0553	0.1483	5.7698	5.9786	0.2088	3.49	2.39±0.96
	2	0.4187	0.1702	5.1249	0.1608	5.8746	5.9783	0.1037	1.73	
	3	0.4209	0.1801	5.1168	0.1445	5.8623	5.9790	0.1167	1.95	
70%	1	0.1914	0.2376	5.0101	0.2308	5.6699	5.9094	0.2395	4.05	3.02±1.46
	2	0.3717	0.2059	5.0447	0.1705	5.7928	5.9102	0.1174	1.99	
	3	-	-	-	-	-	-	-	-	

Table 22C %Weight loss of diazepam submicron emulsions containing Arlasolve DMI after separating by ultracentrifugation.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.4097	0.1706	5.0619	0.1634	5.8056	5.9227	0.1171	1.98	2.78±0.76
	2	0.3147	0.2128	5.0670	0.1792	5.7737	5.9444	0.1707	2.87	
	3	0.3225	0.2095	5.0058	0.2000	5.7378	5.9455	0.2077	3.49	
40%	1	0.3891	0.2184	4.9835	0.1848	5.7758	5.9231	0.1473	2.49	2.16±0.55
	2	0.4076	0.2139	5.0042	0.1722	5.7979	5.9441	0.1462	2.46	
	3	0.3400	0.2107	5.1091	0.1954	5.8552	5.9456	0.0904	1.52	
50%	1	0.3653	0.2035	5.0294	0.1704	5.7686	5.9231	0.1545	2.61	2.45±0.32
	2	0.3996	0.2230	4.9991	0.1663	5.7880	5.9454	0.1574	2.65	
	3	0.5065	0.2004	4.9499	0.1643	5.8211	5.9448	0.1237	2.08	
60%	1	0.3814	0.2080	5.0319	0.1527	5.7740	5.9230	0.1490	2.52	1.44±0.95
	2	0.4255	0.1790	5.0968	0.1807	5.8820	5.9446	0.0626	1.05	
	3	0.4230	0.2210	5.1004	0.1574	5.9018	5.9457	0.0439	0.74	
70%	1	0.4809	0.1813	4.7525	0.1557	5.5704	5.9103	0.3399	5.75	4.44±1.84
	2	0.5047	0.2404	4.8213	0.1570	5.7234	5.9091	0.1857	3.14	
	3	-	-	-	-	-	-	-	-	

Table 23C %Weight loss of lorazepam submicron emulsions containing Arlasolve DMI after separating by ultracentrifugation.

%drug saturated	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
30%	1	0.5507	0.1200	4.8193	0.2217	5.7117	5.9628	0.2511	4.21	2.75±1.50
	2	0.4897	0.1009	5.0731	0.2261	5.8898	5.9624	0.0726	1.22	
	3	0.4476	0.1693	4.9305	0.2469	5.7943	5.9632	0.1689	2.83	
40%	1	0.4153	0.2866	4.8835	0.1331	5.7185	5.9592	0.2407	4.04	3.63±0.37
	2	0.4234	0.2738	4.9148	0.1498	5.7618	5.9591	0.1973	3.31	
	3	0.4892	0.2620	4.8291	0.1676	5.7479	5.9591	0.2112	3.54	
50%	1	0.5370	0.1349	4.8607	0.2602	5.7928	5.9632	0.1704	2.86	2.22±0.86
	2	0.4860	0.1267	5.0458	0.2306	5.8891	5.9630	0.0739	1.24	
	3	0.6279	0.1607	4.7633	0.2578	5.8097	5.9623	0.1526	2.56	
60%	1	0.6135	0.1267	4.9440	0.2197	5.9039	5.9622	0.0583	0.98	1.32±0.61
	2	0.5290	0.1235	4.9192	0.2698	5.8415	5.9626	0.1211	2.03	
	3	0.4356	0.1061	5.0908	0.2688	5.9013	5.9587	0.0574	0.96	
70%	1	0.5639	0.1850	4.7129	0.1642	5.6260	5.9094	0.2834	4.80	3.62±1.66
	2	0.4108	0.1960	5.0008	0.1570	5.7646	5.9095	0.1449	2.45	
	3	-	-	-	-	-	-	-	-	

Table 24C The analytical amount and recovery of alprazolam from submicron emulsion containing Arlasolve DMI after ultracentrifugation.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0.0047	0.0212	0.0585	0.0176	0.1020	6.1343	0.1430	71.36	73.02 ±3.34
	2	0.0048	0.0210	0.0587	0.0254	0.1099	6.1340	0.1430	76.87	
	3	0.0053	0.0219	0.0532	0.0209	0.1013	6.1340	0.1430	70.84	
40%	1	0.0050	0.0290	0.0802	0.0313	0.1455	6.1345	0.1654	87.92	90.68 ±3.83
	2	0.0058	0.0302	0.0950	0.0262	0.1573	6.1350	0.1655	95.05	
	3	0.0056	0.0303	0.0821	0.0293	0.1473	6.1348	0.1654	89.06	
50%	1	0.0099	0.0389	0.0807	0.0321	0.1616	5.9066	0.1909	84.62	85.02 ±2.51
	2	0.0103	0.0359	0.0841	0.0277	0.1579	5.9062	0.1909	82.74	
	3	0.0100	0.0344	0.0929	0.0301	0.1675	5.9070	0.1909	87.72	
60%	1	0.0167	0.0552	0.1485	0.0481	0.2685	5.9072	0.2944	91.20	85.66 ±4.82
	2	0.0140	0.0546	0.1303	0.0435	0.2424	5.9072	0.2944	82.34	
	3	0.0152	0.0511	0.1382	0.0411	0.2457	5.9073	0.2944	83.46	
70%	1	0.0237	0.0577	0.2039	0.0703	0.3556	5.9093	0.3938	90.30	88.18 ±2.99
	2	0.0211	0.0676	0.1753	0.0751	0.3391	5.9108	0.3940	86.06	
	3	-	-	-	-	-	-	-	-	

Table 25C The analytical amount and recovery of clonazepam from submicron emulsion containing Arlasolve DMI afterultracentrifugation.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0	0.0408	0.0468	0.0371	0.1247	5.9764	0.1422	87.68	91.32 ±5.45
	2	0	0.0398	0.0403	0.0459	0.1261	5.9761	0.1422	88.70	
	3	0	0.0391	0.0648	0.0348	0.1388	5.9761	0.1422	97.58	
40%	1	0	0.0599	0.0790	0.0298	0.1687	5.9767	0.1912	88.22	90.32 ±2.68
	2	0	0.0651	0.0565	0.0569	0.1785	5.9765	0.1912	93.34	
	3	0	0.0617	0.0609	0.0484	0.1710	5.9766	0.1912	89.40	
50%	1	0	0.0662	0.0638	0.0649	0.1950	5.9787	0.2091	93.22	92.04 ±1.53
	2	0	0.0649	0.0628	0.0659	0.1936	5.9780	0.2091	92.60	
	3	0	0.0586	0.0689	0.0614	0.1889	5.9783	0.2091	90.32	
60%	1	0	0.1045	0.1024	0.0823	0.2891	5.9786	0.3131	92.34	92.77 ±2.62
	2	0	0.1033	0.0951	0.0847	0.2830	5.9783	0.3131	90.40	
	3	0	0.1091	0.1184	0.0718	0.2993	5.9790	0.3131	95.58	
70%	1	0	0.0335	0.0433	0.0517	0.1285	5.9094	0.1520	84.54	77.93 ±9.35
	2	0	0.0340	0.0432	0.0312	0.1084	5.9102	0.1520	71.32	
	3	-	-	-	-	-	-	-	-	

Table 26C The analytical amount and recovery of diazepam from submicron emulsion containing Arlasolve DMI after ultracentrifugation.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0.9615	0.5048	0.5973	0.3330	2.3967	5.9227	2.4865	96.39	95.47 ±1.81
	2	0.9006	0.6475	0.4869	0.3768	2.4119	5.9444	2.4956	96.64	
	3	0.8144	0.6187	0.5222	0.3756	2.3309	5.9455	2.4961	93.38	
40%	1	1.2352	0.8604	0.6360	0.5106	3.2423	5.9231	3.4120	95.02	99.37 ±4.41
	2	1.4313	0.8632	0.6041	0.4999	3.3986	5.9441	3.4241	99.25	
	3	1.4261	0.9095	0.6979	0.5232	3.5567	5.9456	3.4250	103.84	
50%	1	1.5929	0.9140	0.7665	0.5664	3.8397	5.9231	4.0622	94.52	96.76 ±2.00
	2	1.5078	1.0587	0.8992	0.5458	4.0115	5.9454	4.0775	98.38	
	3	1.6732	0.9360	0.8446	0.5165	3.9704	5.9448	4.0771	97.38	
60%	1	2.0276	1.1276	0.9583	0.6073	4.7209	5.9230	4.8721	96.90	98.00 ±1.51
	2	1.8902	1.0129	1.2962	0.6767	4.8761	5.9446	4.8898	99.72	
	3	2.1007	1.1052	0.9370	0.6199	4.7629	5.9457	4.8907	97.38	
70%	1	2.5988	0.9392	0.7295	0.5456	4.8132	5.9103	5.7229	84.10	85.51 ±1.98
	2	2.4459	1.1214	0.8345	0.5709	4.9728	5.9091	5.7218	86.91	
	3	-	-	-	-	-	-	-	-	

Table 27C The analytical amount and recovery of lorazepam from submicron emulsion containing Arlasolve DMI after ultracentrifugation.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich Ph (mg)	Amount in aq phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
30%	1	0	0.0295	0.0711	0.0896	0.1902	5.9628	0.1984	95.84	95.95 ±0.81
	2	0	0.0251	0.0732	0.0905	0.1888	5.9624	0.1984	95.19	
	3	0	0.0319	0.0705	0.0897	0.1921	5.9632	0.1984	96.81	
40%	1	0	0.0715	0.1021	0.0418	0.2154	5.9592	0.2361	91.23	93.23 ±2.12
	2	0	0.0724	0.0983	0.0546	0.2253	5.9591	0.2361	95.45	
	3	0	0.0674	0.0957	0.0566	0.2197	5.9591	0.2361	93.03	
50%	1	0	0.0518	0.1080	0.1538	0.3136	5.9632	0.3177	98.71	98.40 ±1.00
	2	0	0.0437	0.1203	0.1512	0.3152	5.9630	0.3177	99.21	
	3	0	0.0474	0.1134	0.1483	0.3091	5.9623	0.3177	97.29	
60%	1	0	0.0668	0.1300	0.2273	0.4241	5.9622	0.4544	93.33	93.03 ±0.98
	2	0	0.0615	0.1412	0.2236	0.4263	5.9626	0.4544	93.82	
	3	0	0.0712	0.1263	0.2203	0.4178	5.9587	0.4544	91.94	
70%	1	0	0.1006	0.1799	0.1090	0.3895	5.9094	0.4332	89.91	87.43 ±3.51
	2	0	0.1007	0.1794	0.0879	0.3680	5.9095	0.4332	84.95	
	3	-	-	-	-	-	-	-	-	

Table 28C Percentage of drug distribution into various phases of benzodiazepines containing Arlasolve DMI submicron emulsions

%drug satutared	%drug distribution (Mean±SD)															
	Oil phase				PC rich phase				Aqueous phase				Mesophase			
	A	C	D	L	A	C	D	L	A	C	D	L	A	C	D	L
30%	4.75 ±0.45	0	37.46 ±2.59	0	20.47 ±1.28	30.83 ±1.41	24.82 ±3.26	15.15 ±1.70	54.44 ±2.53	38.76 ±7.44	22.50 ±2.37	37.61 ±1.07	20.34 ±2.93	31.41 ±5.69	15.21 ±1.16	47.24 ±0.63
40%	3.66 ±0.19	0	40.10 ±2.01	0	19.87 ±0.69	36.02 ±0.72	25.84 ±0.61	32.00 ±0.81	57.09 ±2.92	38.04 ±7.89	19.00 ±1.06	44.85 ±0.73	19.38 ±2.47	25.94 ±7.39	15.06 ±0.60	23.15 ±1.32
50%	6.20 ±0.26	0	40.40 ±2.46	0	22.46 ±1.79	32.82 ±0.80	24.59 ±1.56	15.24 ±1.33	52.88 ±2.78	33.89 ±2.25	21.22 ±1.23	36.43 ±1.88	18.46 ±1.24	33.28 ±0.78	13.79 ±0.88	48.32 ±0.62
60%	6.07 ±0.24	0	41.94 ±2.81	0	21.30 ±1.06	36.35 ±0.39	22.62 ±1.64	15.74 ±1.30	55.10 ±1.27	36.19 ±3.07	22.18 ±3.82	31.34 ±1.56	17.53 ±0.69	27.46 ±3.09	13.25 ±0.55	52.92 ±0.60
70%	6.43 ±0.29	0	51.59 ±3.40	0	18.07 ±2.53	29.46 ±2.75	21.03 ±2.15	26.82 ±0.99	54.56 ±4.15	37.69 ±3.06	15.97 ±1.15	47.87 ±1.67	20.94 ±1.60	35.62 ±9.65	11.41 ±0.10	26.16 ±3.02

A = Alprazolam

C = Clonazepam

D = Diazepam

L = Lorazepam

APPENDIX: PART III BENZODIAZEPINE DRUGS

SHAKING METHOD

Table 29C The change in size of benzodiazepine submicron emulsions (nm) prepared by shaking during storage for 7 days at ambient temperature measured by using Photon Correlation Spectroscopy (PCS).

Formulation	Effective diameter (nm)(polydispersity)	
	t=0	t=7
Emulsion base	282.5 (0.149)	285.6(0.097)
Alprazolam	294.3(0.142)	269.0(0.122)
Emulsion base	282.5 (0.149)	285.6(0.097)
Clonazepam	284.7(0.125)	296.3(0.284)
Emulsion base	273.1(0.117)	250.9(0.204)
Diazepam	284.4(0.120)	284.3(0.118)
Emulsion base	273.1(0.117)	250.9(0.204)
Lorazepam	281.9(0.071)	295.6(0.059)

Table 30C The change in size of benzodiazepine submicron emulsions (nm) prepared by shaking during storage for 7 days at ambient temperature measured by using laser light diffraction (Mastersizer 2000).

Formulation	D[4,3] mean (nm)(span)	
	t=0	t=7
Emulsion base	339(1.481)	338(1.526)
Alprazolam	392(1.566)	351(1.516)
Emulsion base	339(1.481)	338(1.526)
Clonazepam	332(1.506)	359(1.647)
Emulsion base	298(1.567)	317(2.414)
Diazepam	305(1.546)	423(1.590)
Emulsion base	298(1.567)	317(2.414)
Lorazepam	304(1.437)	329(1.456)

Table 31C The change in zeta potential of benzodiazepine submicron emulsions prepared by shaking during storage for 7 days at ambient temperature measured by Zetaplus™.

Formulation	Zeta Potential±SD	
	t=0	t=7
Emulsion base	-52.98±5.33	-64.85±2.55
Alprazolam	-17.53±2.14	-46.33±3.63
Emulsion base	-52.98±5.33	-64.85±2.55
Clonazepam	-24.85±1.23	-24.79±1.76
Emulsion base	-52.81±2.08	-21.42±2.63
Diazepam	-16.25±2.27	-28.66±1.66
Emulsion base	-52.81±2.08	-21.42±2.63
Lorazepam	-20.76±3.99	-32.41±3.19

Table 32C The change in pH of benzodiazepine submicron emulsions prepared by shaking during storage for 7 days at ambient temperature.

Formulation	pH±SD	
	t=0	t=7
Emulsion base	5.68±0.04	5.15±0.02
Alprazolam	5.95±0.02	5.38±0.05
Emulsion base	5.68±0.04	5.15±0.02
Clonazepam	5.23±0.07	5.27±0.05
Emulsion base	5.37±0.03	5.18±0.006
Diazepam	5.62±0.02	5.18±0.03
Emulsion base	5.37±0.03	5.18±0.006
Lorazepam	5.62±0.04	5.65±0.006

Table 33C %Weight loss after separating by ultracentrifugation of benzodiazepines incorporated in submicron emulsions by shaking.

drug	n	Oil phase weight (g)	PC rich phase weight (g)	Aqueous phase weight (g)	Mesophase weight (g)	Total weight (g)	Initial weight of SME (g)	Losing weight (g)	% loss	Mean±SD
Alprazolam	1	0.4385	0.1926	4.7450	0.3136	5.6897	5.8403	0.1506	2.58	1.8±0.87
	2	0.4766	0.2159	4.7911	0.2423	5.7259	5.8401	0.1142	1.96	
	3	0.5139	0.1609	4.8916	0.2240	5.7904	5.8409	0.0505	0.86	
Clonazepam	1	0.3104	0.1930	4.2779	0.2401	5.0214	5.0638	0.0424	0.84	0.84±0
	2	-	-	-	-	-	-	-	-	
	3	0.3204	0.1894	4.3120	0.1995	5.0213	5.0639	0.0426	0.84	
Diazepam	1	0.3582	0.2037	4.0792	0.2622	4.9033	5.0632	0.1599	3.16	1.76±1.27
	2	0.3060	0.1713	4.2644	0.2500	4.9917	5.0638	0.0721	1.42	
	3	0.2421	0.2134	4.3292	0.2440	5.0287	5.0635	0.0348	0.69	
Lorazepam	1	0.4296	0.1218	4.9555	0.3031	5.8100	5.8406	0.0306	0.52	1.34±1.33
	2	0.5143	0.0565	4.9317	0.3015	5.8040	5.8401	0.0361	0.62	
	3	0.6034	0.0659	4.6539	0.3501	5.6733	5.8410	0.1677	2.87	

Table 34C The analytical amount and recovery after ultracentrifugation of benzodiazepine incorporated in submicron emulsion by shaking.

%drug saturated	n	Amount in oil phase (mg)	Amount in PC rich phase (mg)	Amount in aqueous phase (mg)	Amount in mesophase (mg)	Total amount (mg)	Initial weight of SME (g)	Theoretical amount (mg)	%Recovery	Mean±SD
Alprazolam	1	0.0033	0.8609	1.2808	1.7108	3.8558	5.8403	4.0228	95.85	97.84± 1.72
	2	0.0198	0.6642	0.9039	1.0849	2.6728	5.8401	2.7065	98.75	
	3	0.0068	0.4301	1.0024	0.7727	2.2120	5.8409	2.2363	98.91	
Clonazepam	1	0.2037	0.3273	0.3201	0.3759	1.2270	5.0638	1.6255	75.48	77.77± 3.24
	2	-	-	-	-	-	-	-	-	
	3	0.1503	0.3219	0.3229	0.3731	1.1682	5.0639	1.4591	80.06	
Diazepam	1	2.4722	2.8876	1.1228	2.0215	8.5041	5.0632	9.3666	90.79	93.32± 7.95
	2	2.7472	2.2650	1.2969	2.1693	8.4784	5.0638	9.7522	86.94	
	3	2.3220	2.5166	1.6131	2.3426	8.7943	5.0635	8.6033	102.22	
Lorazepam	1	0.2271	0.9438	1.0040	3.7163	5.8912	5.8406	5.7321	102.77	100.83± 4.42
	2	0.2889	0.2636	1.4631	4.2625	6.2781	5.8401	6.0402	103.94	
	3	0.2480	0.3438	1.2046	4.6155	6.4119	5.8410	6.6954	95.77	

Table 35C Percentage of drug distribution into various phases of submicron emulsion by shaking

Drug	% Drug distribution (Mean±SD)			
	Oil phase	PC rich phase	Aqueous phase	Mesophase
Alprazolam	0.38±0.33	22.21±2.70	37.45±6.82	39.96±4.75
Clonazepam	14.73±2.64	27.11±0.62	26.86±1.09	31.29±0.92
Diazepam	29.29±3.00	29.76±3.75	15.61±2.58	25.33±1.45
Lorazepam	4.11±0.43	8.53±6.52	19.71±3.23	67.65±4.46

Table 36C Effect of incorporated methods on the distribution of alprazolam into various phases of submicron emulsions.

Method of incorporation	% Drug distribution (Mean±SD)			
	Oil phase	PC rich phase	Aqueous phase	Mesophase
De novo emulsification (70% saturated oil solubility)	3.84±0.35	38.22±2.87	39.50±0.57	18.43±2.15
Extemporaneous addition (70% saturated oil solubility)	0±0	18.07±2.53	60.96±4.57	20.94±1.60
Shaking	0.38±0.33	22.21±2.70	37.45±6.82	39.96±4.75

Table 37C Effect of incorporated methods on the distribution of clonazepam into various phases of submicron emulsions.

Method of incorporation	% Drug distribution (Mean±SD)			
	Oil phase	PC rich phase	Aqueous phase	Mesophase
De novo emulsification (70% saturated oil solubility)	3.10±0.93	48.70±12.40	30.63±2.08	17.57±11.54
Extemporaneous addition (70% saturated oil solubility)	0±0	29.46±2.75	37.69±3.06	35.62±9.65
Shaking	14.73±2.64	27.11±0.62	26.86±1.09	31.29±0.92

Table 38C Effect of incorporated methods on the distribution of diazepam into various phases of submicron emulsions.

Method of incorporation	% Drug distribution (Mean±SD)			
	Oil phase	PC rich phase	Aqueous phase	Mesophase
De novo emulsification (70% saturated oil solubility)	50.30±2.85	28.09±1.26	11.67±1.93	9.94±1.58
Extemporaneous addition (70% saturated oil solubility)	51.59±3.40	21.03±2.15	15.97±1.15	11.41±0.10
Shaking	29.29±3.00	29.76±3.75	15.61±2.58	25.33±1.45

Table 39C Effect of incorporated methods on the distribution of lorazepam into various phases of submicron emulsions.

Method of incorporation	% Drug distribution (Mean±SD)			
	Oil phase	PC rich phase	Aqueous phase	Mesophase
De novo emulsification (70% saturated oil solubility)	2.53±0.29	38.74±0.60	35.29±1.42	23.91±0.22
Extemporaneous addition (70% saturated oil solubility)	0±0	26.82±0.99	47.87±1.67	26.16±3.02
Shaking	4.11±0.43	8.53±6.52	19.71±3.23	67.65±4.46

VITA

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