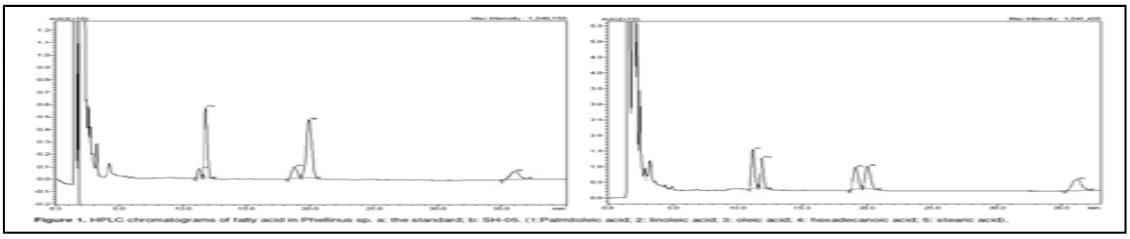
1. Fatty acids: Palmitic acid, Olic acid, Myristic acid, Stearic acid

Fatty acid analysis by HPLC using PDA detector

Material and method:

- **Plant materials** :The Plant materials were collected and conserved
- **Chemicals and reagents**: Palmitic acid, Myristic acid, oleic acid , Stearic acid were purchased. HPLC grade acetonitrile was used for the HPLC analysis. Deionized water was purified. The acetic acid and CTAB were of AR grade, purchased.
- **Apparatus and chromatographic conditions:** HPLC instrument equipped with a quaternary pump, a photo diode-array detector and a column compartment was used. The sample was separated on **a Hyperchrome C18 column** (5µm, 4.6×250 mm). The mobile phase consisted of acetonitrile and 5%CTAB water (95:5,v/v) . HPLC separation was performed at 20℃ and a flow rate of 1.5 ml/min. **PAD detector** was set to scan from 200 to 800 nm, and 242 nm was used as detection wavelength for analysis.
- **Sample preparation:** Fresh Herbs (1 g) were ground in liquid nitrogen, and 2 ml chloroform, 1 ml 1mol/L sodium hydroxide-methanol were added and saponified at room temperature for 45 min. The extract was suspended in 1 ml2 mol/L hydrochloric acid-methanol and vibrated for 2 min. After 10 min, 1.8 ml water was added and subnatant was dehydrated by anhydrous sodium sulfate. Aliquot (400 µl) of the extracted lipids was dried under nitrogen and resuspended in 100 µl 2-bromocacetophenone (10 mg/ml in acetone). After vortex mixing, 100 µl trithylamine (10 mg/ml in acetone) was added. The mixture was sealed immediately in a screw capped glass tube and heated for 5 min in a boiling water bath. After cooling, 160 µl acetic acid (2 mg/ml in acetone) was added and the tube was heated for an additional 5 min. The resulting FAPEs were filtered through a 0.22 µm microfilter membrane and redissolved in 500 µl methanol for HPLC injection.
- **Results and Discussion**

  Identification of fatty acid compounds was carried out by comparing HPLC retention timesand UV absorptions.



2. Fatty Alcohols: Lauryl alcohol, Cetostryl alcohol

Fatty alcohol analysis by HPLC using PDA Detector

Material and method:

In this study, high-performance liquid chromatography (HPLC) in conjunction with continuous derivatization for the determination of aliphatic and polyethoxylated alcohol is reported. Reaction of alcohol group with phenyl isocyanate or benzyl chloride reagents assisted with microwaves (MW) irradiation is carried out in an on-line system coupled to HPLC with **photodiode array detection (PDA).** Reactor was placed into a microwave oven at 450 W. The flow rate, reagent amounts, irradiation time, and chromatographic conditions were optimized. The continuous analysis using the system **MW-HPLC-PDA** provided high sensitivity, reduce the amount of reagents and analysis time. This proposed method can be used for the analysis of commercial alcohol polyethoxylated mixture.

3. Glycols: Ethylene glycol and Polyethylene alcohol

Glycols analysis by HPLC using PDA Detector

Material and Method:

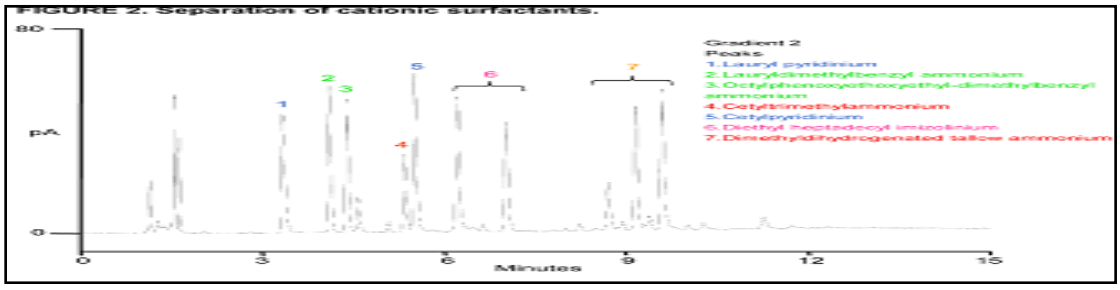
A high-throughput, high performance liquid chromatographic method was developed and validated for the determination of clopidogrel in pharmaceutical dosage forms. The analysis was performed at room temperature using a reversed phase monolithic silica column **hyperchrome**. The mobile phase consisted of acetonitrile:phosphate buffer (50 : 50 *v/v*, pH 3.0) at a flow rate of 4.0 mL/min. **The photodiode array detector** was set at 235 nm. The developed method showed a good linear relationship in the concentration range from 1.0 to 40.0 µg/mL with a correlation coefficient of 0.999. The limit of detection and limit of quantification were 0.97 µg/mL and 3.52 µg/mL, respectively.

4. Non ionic Surfactant of Ethoxylate: Nonyl phenol ethoxy group

Ethoxylate non ionic surfactant analysis by HPLC using PDA detector

Material and method:

- **Separation Column**
  All separations were performed on Ethoxylate Surfactant Plus Hyperchrome column (3 µm, 3 × 150 mm).
- **Samples**
  All surfactant standards were purchased. All consumer products were purchases from local stores. Samples were dissolved in a isopropanol, isopropanol/water (1:1), or acetonitrile/water (1:1) to a concentration of 1 to 20 mg/mL, and filtered before injection.
- **Mobile Phase System**
  The mobile phase contained acetonitile and 0.1 M ammonium acetate (pH5) buffer.
  HPLC grade acetonitrile was used. Deionized water (>18MΩ-cm) was purified by water purification system. Ammonium acetate salt (99.99+% pure), Glacial acetic acid. The buffer consists of 7.78 g/L ammonium acetate and 2.05 g/L glacial acetic acid in water.
- **HPLC Instrument**
  Separations were performed on a binary HPLC system equipped with **PDA detector**.
  Scattering detector was used to detect analytes with no or weak chromophore.
- **Discussion and Results**
  Separation Column
  Hyperchrome HPLC column designed for the determination of surfactants, including anionics, nonionics, cationics, and amphoterics, in a wide range of samples, such as consumer products, pharmaceuticals, food & beverages, environmental samples, etc. This column, based on novel mixed-mode chromatography technology and advanced surface chemistry, provides both reversed-phase and anion-exchange retention mechanisms. The column chemistry is designed in such way that it elutes in the order of cationic, nonionic, amphoteric, and anionic surfactants.

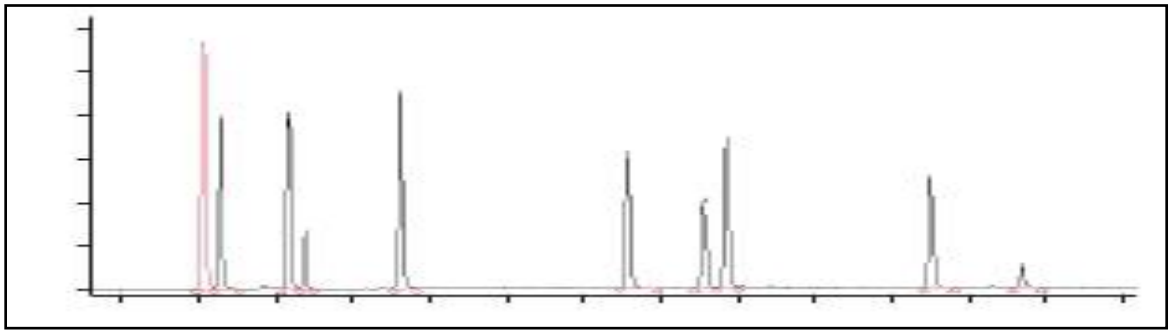


5. Acrylate monomers: Styrene monomer, Butyl acrylate, Ethyl acrylate

Acrylate monomers analysis by HPLC-PDA:

Material and method:

- **Reagents:** Analytical-grade acetonitrile (MeCN), hydrochloric acid 37% (HCl) and tetrahydrofuran (THF), HPLC-grade water(H2O), acetonitrile and Ultrapur o-phosphoric acid (85%).Water for solutions was deionized before use. The (meth)acrylic monomers were obtained from several manufacturers. To avoid contamination, the HPLC-grade water and acetonitrile were taken directly,without filtering, from 2.5 L bottles to which 0.1% Ultrapur o-phosphoric acid (85%) was added.
- **Chromatography:** Hyperchrome column was used.The flow-rate was 0.7 mL/min at room temperature. The reservoirs containing MeCN and H2O, to which 0.1% Ultrapure o-phosphoric was added, were purged with 50 mL/min helium to remove dissolved air, which otherwise caused problems with the gradient. The gradient was 100% H2O to MeCN in 30 min for standards. For samples and some apolar (meth)acrylic monomers it was necessary to hold the system for 15 min on 100% MeCN. Between two gradient cycles 20 min were taken for column equilibration. The column performance didn't change significantly,under these working conditions, in a time period of one year. The injection volume was 10 µL and after separation and detection, the peaks were integrated and calibrated Software, using the external standard.
- **Preparation of standards**: Every week new stock solutions (10 mg/mL) were prepared by weighing approximately 100 mg of each (meth)acrylated monomer on an analytical balance in separate 10 mL volumetric flasks and diluting to the mark with MeCN. Dilutions of the stock were made at every new series of measurements. The concentrations of these dilutions, into the same volumetric flask, were 1000 (for recovery), 100, 25, 5 and 2 µg/mL (for the calibration curve).There were some exceptions to the procedure of making standards. For very polar monomers, such as acrylic acid,acrylamide and hydroxyethylacrylate, it was necessary to make the solutions in H2O/MeCN 9/1. When the solutions were too rich on MeCN (>10 vol. %), the peaks for these monomers became bimodal and were, therefore, difficult to integrate. For (meth)acrylic acid, the addition of o-phosphoric acid to the eluent was necessary. This prevented the dissociation of the acid and the peak became more symmetrical. Furthermore, it was necessary to adjust the concentrations of some standards, to hold them between normal ranges of UV absorption (0.1–1.0 aufs).



6. Amines: Triethanolamine, Diethanolamine

Amines analysis by HPLC- PDA

Material and method:

- **Chemicals**
  Benzoyl chloride, DFMA
- **Plant Material**
  Herbal plants were grown under standard greenhouse conditions in vermiculite. Mature leaf tissues from 2 month old plants were homogenized at 100 mg fresh weight tissue/mL of 5% PCA on ice. After 30 min, the extracts were centrifuged for 10 min at 27,000g. The supernatant was then acid-hydrolyzed in 6 NHCI for 18 h at 1 10°C. The hydrolysate was dried under a stream of air at 80°C, then resuspended in PCA prior to derivatization.
- **TLC Analysis of PAs**
  Amine samples were dansylated, separated on Whatman LK6D high-performance silica TLC plates, and quantitated.
- **HPLC Analysis of PAs**
  Polyamine standards and unknowns were benzoylated. HPLC analysis of benzoyl-PAs was performed using a programmable Hyperchrome C18 reverse-phase column using a Benzoyl PAs were eluted at a flow rate of 1.0 mL/min using one of two water (solvent A)/MeOH (solvent B) stepped gradient programs followed by a column cleaning/regeneration cycle.

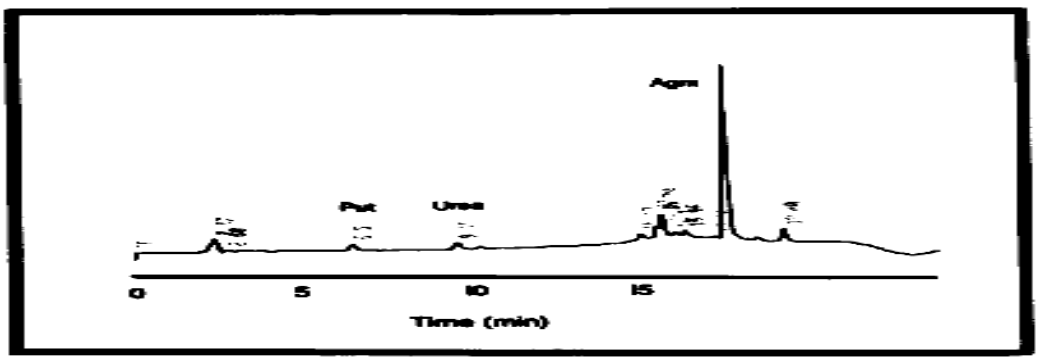


Figure 3. HPLC elution profile for agmatine standard (5 nmol) benzoylated at pH 9 for 30 min, 25°C and chromatographed as in Figure 1. Note approximate stoichiometric amounts of the Put and urea degradation products (~0.46 nmol each).

7. Fatty oil: Soybean oil, Castor oil

Fatty oil analysis by HPLC-PDA

Material and method:

- **Testing Materials**

  Soybean seeds (SB).Soybean seeds were ground to fine soybean flour using a commercial blender and the soy flour was kept in sealed plastic bags stored at -20^{0}C freezer.

- **Reagents**

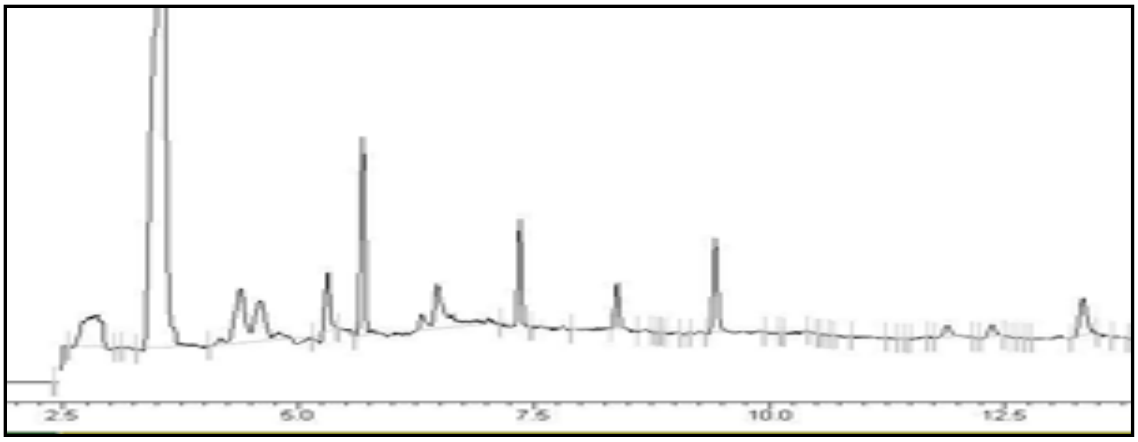
  Sodium hydroxide(analytical grade), Citric acid (analytical grade), hexanes (HPLC grade), methanol (HPLC grade), ethyl acetate (HPLC grade), BCL3-methanol, 98% 2, 2-Dimethoxypropane, Anhydrous sodium sulfate (10-60 mesh), cholesterol, 5α-cholestane , heptadecanoic acid , (+)-γ-tocopherol , glyceryl trioleate, triglyceride, DHA (cis-4, 7, 10, 13, 16, 19-Docosahexaenoic acid. The solvents were stored at room temperature (20-25^{o-}C) and other reagents were stored at -20^{o}C freezer. Sodium Hydroxide and citric acid were dissolved in distilled water. All of organic reagents were dissolved in hexanes, except for being particularly noted.

  Chromatographic silica gel (70-230 mesh, grade 62) was purchased.Disposable culture tubes, 18 Whatman filter papers.

- **The Fractionation of the Fraction of silica gel Using RE-HPLC**

  The eluent2 was eluted and fractionalized by RE-HPLC using a scheme of two-step elation, at 20^{o}C room temperature. HPLC system was Water 2690 separation module, consisting of pumps, autosampler, injector, and **photodiode array detector**, equipped with a Hyperchrome column (250cm × 4.6mm, 5µ of particle size. The pressure limits was set as 4000 PSI. Pump mode was isocratic with a flow rate of 1ml/min. 996 **PDA** was set as a scanning range of wavelength of 190-700nm with resolution of 1.2nm. The wavelength of display on monitor was set as 204nm when running samples.

At the first-step of the elution, pure methanol was delivered as the mobile phase. The run time was 20min. Based upon the analyses for the HPLC chromatograms of the sample, afterwards, the eluent were arbitrarily fractionalized into two parts: the fractions of 0-10min and 10-20min, collected combined in centrifuging tubes, respectively. The operation of fractionalization was performed 20 times (20 circles). The two fractions gained were evaporated by a rotary vacuum evaporator to reduce the volume and increase the concentrationsrespectively. The final volumes of each fraction were set as 10ml. Each fraction was analyzed for antioxidant activity by the cholesterol model to determine a fraction with the bigger activity respectively. At the second-step of the elution, a fraction determined with bigger activity at the previous step was run with a M.P. of 5%water in methanol, where the run time was 12min. Based upon the analyses for the HPLC chromatograms of the run sample, the eluent was arbitrarily fractionalized into two fractions: 0-6min fraction and 6-12min fraction, collected in centrifugation tubes, respectively. The fractionalization operation was run 20 times (20 circles). Each fraction was evaporated to reduce volume to less than 5 ml. The final volumes of each fraction were set as 5ml. The two fractions were analyzed for antioxidant activity by the cholesterol model to determine a fraction with bigger activity.



8. Ethoxylated alcohol impurities analysis

Material and method:

Materials

PEG standard and 2,5-dihydroxy benzoic acid (DHB), HPLC grade methanol and deionized water, All FAEs, denoted as $C_{18}EO_{10}$ (stearyl alcohol ethoxylates with an average EO = 10), $C_{18}EO_{20}$ (stearyl alcohol ethoxylates with an average EO = 20), $C_{16}EO_{10}$ (cetyl alcohol ethoxylates with an average EO = 10), $C_{16}EO_{20}$ (cetyl alcohol

ethoxylates with an average EO = 20), C_9EO_{10} (oleyl alcohol ethoxylates with an average EO = 10), and $C_2C_{14}EO20$ (isohexadecyl alcohol ethoxylates with an average EO = 20). FAE samples were prepared for analysis by dissolving in methanol/water 80/20 (V/V) mixture. The concentrations were approximately 0.1 g mL^{-1}.

Chromatography

The chromatographic system consisted of a Waters 600E pump with a controller, a injector, and a detector. The ELSD detector was set with the nebulization air pressure at 2.5 bar and the heating temperature at 40 ◦ C. The column was **Hyperchrome** C1 (4.6 mm × 150 mm). Mobile phase A was a mixture of H2 O/methanol 80/20 (V/V). Mobile phase B was methanol. The flow rate was 1.0 mL min−1 .

MALDI

The mass spectra were obtained with a mass spectrometer. The spectrometer was operated in the reflector mode. The accelerating voltage was +20,000 V and the grid volt- age was 76%. The delayed extraction time was 100 ns. The laser (N_2 , 337 nm) intensity was 2200 in the range of 0–2400 scale. The spectra are averages of 100 laser shots.Ten milligram dihydroxybenzoic acid and 1 mg of NaCl were dis-solved in 2.0 g of a methanol/H_2O mixture (50/50, V/V) to form the matrix solution. Approximately 15 mg of the matrix solution was added to the collected LC fraction and the fraction was evaporated to1/3 of its original volume. One microliter of the concentrated sample fraction was deposited on the gold plate and dried at ambient conditions in preparation for MALDI-TOF MS analysis.

Results and discussion

Separations

The major impurity expected to be present in the FAEs is PEG with hydroxyl end groups (denoted as free PEG). Initial studies with a C18 column resulted in long analysis times (results not shown). A C1 column was subsequently selected because it has been previously demonstrated to have the highest peak capacity in the isocratic mode separation of oligomeric components of PEG. In this study, isocratic elution is desired for the purpose of quantitation. Fig. 1 shows the separation of $C_{18}EO_{10}$ using three initial mobile phase compositions—60, 64, and 68% methanol in water. Isocratic elution was employed in the first 10 min to separate impurities from the void peak. This was followed by gradient elution to a final mobile phase composition of 88% methanol. The ELSD sensitivity was set at an optimum level to detect the elution of the low-concentration impurities and thus the elution of FAEs registered as a large off-scale peak. The initial mobile

phase of 60% methanol in water yielded a major peak between 20 and 25 min that represents the elution of ethoxylated stearyl alcohols. In addition, the low-intensity oligomeric peaks observed at early retention.