FP7-KBBE-2010-4 265942

The sustainable improvement of European berry production, quality and nutritional value in a changing environment: Strawberries, Currants, Blackberries, Blueberries and Raspberries.

Work Package nr.3

FRUIT QUALITY CHARACTERIZATION AND DETERMINATION

Deliverable 3.1

Delivery of Standard Procedure of analitycal approaches

INDEX

METHODOLOGIES P1

For fruit sensorial and nutritional analysis as prescreening tools for genotype evaluation.

FRUIT BIOACTIVE COMPOUNDS EXTRACTION METHOD

- 10g of fruit is weighed and used for the extraction.
- Strawberry: the extraction takes place in a solution of methanol and water (80% v/v) added to the pieces of strawberry in ratio of 1:5 (1 part of fruit:5 part of extraction phases, 10 g of fruits in 50 ml of extraction phase)
- Blueberry and Raspberry: the extraction takes place in a solution of acidified methanol (1% glacial acetic acid on extra volume methanol). The extraction phase is added to the pieces of strawberry in ratio of 1:5 (1 part of fruit:5 part of extraction phases, 10 g of fruits in 50 ml of extraction phase)
    - First extraction with 20ml of methanol, extraction phase
    - Homogenize the mixture, it has to be placed in continuous agitation (or ultrasound assisted) along 30 minutes. The extraction has to be in dark (cover the falcon tube with aluminum foil).
    - Separate the solid phase from the liquid phase by centrifugation at 4500g for 10 min.
    - Recover the supernatant and stock it in a new falcon tube by a glass Pasteur pipette.
    - Second extraction adding 20ml of methanol in the falcon where are placed the grinded fruit that have been extracted a first time yet.
    - Homogenize the mixture, it has to be placed in continuous agitation (or ultrasound assisted) along 30 minutes. The extraction has to be in dark (cover the falcon tube with aluminum foil).
    - Separate the solid phase from the liquid phase by centrifugation at 4500g for 10 min.
    - Recover the supernatant by a glass Pasteur pipette and stock it in the falcon tube where was placed the supernatant from the first extraction.
    - Transfer with a glass pipette the supernatant from the falcon tube to vials and store in freezer at -20°C.

For this type of extraction the determination of Anthocyanin content has to take place immediately after extraction.

TOTAL ANTIOXIDANT CAPACITY (CAT)

by Trolox Equivalent Antioxidant Capacity method (TEAC)

Intention

The pre-formed blue/green radical of $ABTS^{\bullet+}$ is generated by oxidation of ABTS with potassium persulfate. The radical cation has an absorption maximum at 734 nm. It is reduced in the presence of such hydrogen-donating antioxidants. The decolorization of the $ABTS^{\bullet+}$ radical is determined as a function of concentration and calculated relative to the reactivity of Trolox, a water-soluble vitamin E analogue, as a standard under the same condition (Miller et al.,1993; Re et al., 1999) .

Material

Equipment

Photometer

Plastic Cuvette 1 cm

Stopwatch

Ultrasonic bath/ Shaker

Chemicals

- ABTS (2,2´-azinobis
- Trolox (6-hydroxy-2,5,7,8 tetramethychroman-2-carboxylic acid)
- Potassium persulfate (di-potassium peroxidisulfate)
- Dipotassium hydrogen phosphate
- Potassium dihydrogen phosphate
- Ethanol
- PhosphaTe buffered saline (**PBS**, 5mM, pH 7,2 - 7,4) 7,14 g (41 mmol/L) of dipotassium hydrogen phosphate (K_2HPO_4) and 1,23 g (9 mmol/L) Potassium dihydrogen phosphate (KH_2PO_4) filled up with water to 1 L.
- ABTS stock solution 77 mg ABTS are resolved in a 20 mL volumetric flask with a few ml PBS. 13 mg Potassium persulfate are weighed in a beaker and equally resolved with PBS (Ultrasound assisted!), before added to the ABTS. The flask is filled up with PBS to its mark. Before use it is necessary that the mixture stays in the dark (aluminium foil) at room temperature for 12 to 16 hours (at night). The solution is stable in the dark for five days.
- ABTS working solution. The ABTS stock solution has to be diluted with PBS, then filtered wit a paper filter, to an absorbance of 0,7 – 0,8. (1:50 to 1:70).

- Trolox stock solution. 32 mg Trolox is weighed in a 50 ml volumetric flask and resolved with a few ml ethanol and filled up with PBS to its mark (2,5mM).

- **Procedure**
  - Sample preparation

See extraction methodology. Supernatant is diluted 1:20 (100µL sample: 2000µL).

  - Measuring

At first transfer by pipette 1900 µL of ABTS working solution into the cuvette. The reaction starts after addition of the sample solution respectively blank or standard (100 µL) and should be mixed immediately. The absorbance of the sample is measured after 6 minutes at 734 nm.

	Sample	Blank
ABTS Working Solution	1900 µL	1900 µlL
PBS	-	100 µL
Sample solution	100 µL	-
Total	2000 µL	2000 µL

  - Calibration

The Trolox stock solution is diluted with PBS so that the final concentration of the dilution series ranges from 0,025 to 0,450 mmol/L. The Trolox solutions are measured like sample 3.2.

Calculation

To obtain the percentage of inhibition:

$$\%inhibition = \frac{Abs_{blank} - Abs_{sample/standard}}{Abs_{blank}} \times 100\%$$

The calibration is calculated by linear regression (Δ A = ac + b, c = concentration trolox mmol/l, Δ A = %inhibition, a = %slope, b = %intercept).

$$TEAC - Value\ (mg\ Trolox\ eq/kg\ Fruit) = \frac{(\Delta A - b) \times F}{a \times E}$$

Δ A = %inhibition

a = slope

b = intercept

F = Dilution factor (20)

E = sample weight [kg/L extracting agent]

- **Results**

TEAC-Value is expressed as [mmol Trolox equivalent/ kg] fruit with one decimal accuracy.

Attention: The TEAC-Value comprehends the antioxidative capacity of ascorbic acid.

TOTAL ANTIOXIDANT CAPACITY (CAT)

by Ferric reducing antioxidant power (FRAP).)

Intention

Antioxidant capacity of the sample solution is determined by its ability to reduce ferric to ferrous iron. When iron is complexed with 2,4,6-tripyridyl-s-triazine (TPTZ) in sodium acetate solution at an acidic pH, its reduction results in a colour change of the solution, from pale rust to blue. The absorbance of the solution at 593 nm reflects the extent of reduction. The reducing power is usually compared to that of ferrous sulphate, but other aqueous antioxidants, such as the vitamin E analogue Trolox are used as alternative standards.

Material

Chemicals

- Sodium acetate trihydrate 300mM Solution: dissolve 3.1 g sodium acetate in 950 ml H_2O, adding 16 ml glacial acetic acid. Bring total volume to 1000ml. Store in glass bottle at room temperature.
- Ferric chloride hexahydrate 20mM solution: dissolve 0.270 g ferric chloride hexahydrate in H_2O, total volume 50 ml. This reagent must be prepared fresh immediately prior to procedure.
- 2,4,6-tripyridyl-s-triazine (TPTZ) 10mM solution: add 340 μl concentrated HCl to H_2O, total volume 100ml. Dissolve 0.312 g TPTZ in HCl solution with stirring. Store in dark glass bottle at room temperature.
- Hydrochloric acid
- Ferrous ammonium sulphate or Ferrous sulphate heptehydrate: dissolve 0.0098 g ferrous ammonium sulphate hexahydrate (mol wt 392.1) in 25 ml H_2O (1000 μM stock). (Alternatively can use ferrous sulphate heptahydrate (mol. Wt 278.0), 0.0278 g in 100ml H_2O) to make the 1000 μM stock. The stock and dilutions should be prepared fresh prior to performing procedure

  Dilute appropriately to prepare remaining standards:

  500 μM std = 500 μl of 1000 μM stock + 500 μl H_2O
  200 μM std = 400 μl of 500 μM std + 600 μl H_2O
  150 μM std = 150 μl of 500 μM std + 350 μl H_2O
  100 μM std = 500 μl of 200 μM std + 500 μl H_2O
  50 μM std = 500 μl of 100 μM std + 500 μl H_2O
  0 μM std = H_2O

- Trolox Standards: dissolve 0.0624 g Trolox in 25 ml methanol (10 mM stock; this may be frozen for up to 6 months). These should be prepared fresh from the frozen stock prior to performing procedur

Additional dilutions are made as follow:

500 μM std = 50 μl of 10 mM stock + 950 μl H_2O
250 μM std = 500 μl of 500 μM std + 500 μl H_2O
100 μM std = 500 μl of 250 μM std + 750 μl H_2O
75 μM std = 300 μl of 100 μM stock + 100 μl H_2O
50 μM std = 500 μl of 100 μM stock + 500 μl H_2O
25 μM std = 500 μl of 50 μM stock + 500 μl H_2O
0 μM std = H_2O

- Milli-Q water for all reagent preparation
- FRAP reagent**: c**ombine 10X volume of sodium acetate solution with 1X volume TPTZ and 1X volume of ferric chloride solution.

This reagent must be prepared fresh immediately prior to procedure.

Equipment

- Spectrophotometer
- Plastic Cuvette 1 cm (plastic)
- Volumetric flasks
- Falcon tube

Procedure

Prepare appropriate dilutions of extracts to be analyzed in H_2O. 100 μl of the appropriate dilution is needed for each sample tested in singlet

Prepare ferrous sulphate and Trolox standards, and hold at room temperature.

Prepare ferric chloride solution; then use the sodium acetate solution, TPTZ solution and ferric chloride to prepare the FRAP reagent, as introduced above. This reagent is stable for at least 2 hours at room temperature.

For reading in non automated spectrophotometer, pipette 900 μl FRAP reagent into cuvette (acrylic disposable are OK), followed by 100 μl EXTRACT SAMPLE or STANDARD and mix quickly by pipetting up and down (start timer immediately after addiction). The most complete and efficient mixing occurs when the tip is held just under the surface of the mixture in the cuvette, with rapid aspiration and expulsion. Blanks for zeroing the spec are prepared by adding 100 μl H_2O to 900 μl FRAP reagent, unless interference from the extraction solvent is suspected (doesn't occur with methanol or ethanol/water when diluted as above). Note thet the blank can be used as the zero standard, unless an adjustment for solvent is necessary.

Zero the spec with the blank at 593 nm and then EXACTLY 4 minutes following the addition of the samples to the FRAP reagent, read the absorbance.

Raw values of the FRAP are determined from the respective standard curves. The Trolox curve provides values in mmoles Trolox equivalent (TE), while the ferrous sulphate provides them in ferric reducing equivalents.

Results

For final values on a mmoles (TE or ferric reducing equivalents)/Kilogram of Fresh Weight

$$\frac{\text{raw FRAP value X dilution factor (e.g. 100) X volume of solvent extract (e.g. 100ml)}}{\text{wt (g) of fruit sample (e.g. 20 g berries) X } 10^3}$$

The ratio of ferrous sulphate/Trolox values on a molar basis is usually about 2:1. It's requires twice as many moles ferrous sulphate to achieve the same reducing power as Trolox.

TOTAL PHENOL CONTENT

by Folin Ciocaltou's reagent method

Intention

The total phenolics assay does not only determine phenolics but also reducing agents like ascorbic acid, because the basic mechanism is an oxidation/reduction reaction. The exact chemical nature is not known, but it is believed to contain heteropolphospho-tunstates molybdates. Molybdenum seems to be easily reduced in the complex. An electron-transfer reaction occurs between reductants and Mo(VI) under alkaline conditions, which results in blue color with an absorbance maxima about 720 nm.

- **Material**

Equipment

- Spectrophotometer
- Plastic Cuvette 1 cm (glass, plastic, quartz??)
- Stopwatch

Chemicals

- Folin-Ciocalteu-Reagent
- Sodium carbonate
- Gallic Acid
- Sodium carbonate solution 20% 200 g sodium carbonate is filled up with water to 1 L.
- Stock solution: 1000 Gallic Acid mg /L 200mg. Gallic Acid is solubilized in a few drops of Methanol to avoid the formation of agglomerate hard to resolve, and filled up with water to 200 mL.
- Standard: Gallic Acid. The Gallic Acid stock solution is diluted with water so that the final concentration of the dilution series ranges from 10 to 50 mg Gallic Acid/L (0,1ml; 0,2ml; 0,3ml; 0,4ml; 0,5ml in 10ml volumetric flask)

Procedure

- Sample preparation.

See extraction methodology. Supernatant is diluted 1:20 (100µL sample: 2000µL).

- Measuring

A test tube (glass) is filled with 7.0 ml water. Afterwards 1 mL of the diluted sample (only water is used for the blank measurement) is added which is followed by 500 µL Folin-Ciocalteu-Reagent an

vortexed. After 3 minutes 1,5 mL sodium carbonate is added and the tube is mixed one more time. The absorbance of the sample is measured after exactly 60 minutes at 760 nm.

- Calibration

The Gallic Acid standards are measured like sample 3.2. The calibration has to be repeated when a new Folin-Cioclateu reagent is used.

Calculation

The calibration is calculated by linear regression (Δ A = ac + b, c = concentration Gallic Acid mg/l, Δ A = absorbance, a = slope, b = intercept).

$$TP(mg\ GallicAcid\ eq/kg\ Fruit) = \frac{(\Delta A - b) \times F}{a \times E}$$

Δ A = $A_{sample/standard}$

a = slope

b = intercept

F = Dilution factor (20)

E = sample weight [kg/L extracting agent]

- **Results**

TP is expressed as [mg Gallic Acid equivalent/ kg] fruit without decimals.

TOTAL ANTHOCYANIN CONTENT

by pH Differential Shift Method

Intention

Anthocyanin pigments change hue and intensity according to pH. At pH 1.0, anthocyanins exist in the colored oxonium or flavylium form and at pH 4.5 predominantly in the colorless carbinol form. One aliquot of an aqueous anthocyanin solution is adjusted to pH 1.0 and another aliquot to pH 4.5. The difference in absorbance is proportional to the anthocyanin content. Determination of anthocyanin content is based on Lambert-Beer's Law. Published Molar absorbance values for purified pigments are used, making determination unnecessary . Pelargonidin-3-glucoside is the major anthocyanin in Strawberry, so the total anthocyanin content is calculated as pelargonidin-3-glucoside.

Material

Equipment

- Spectrophotometer
- Plastic Cuvette 1 cm (glass, plastic, quartz??)
- Volumetric flasks

Chemicals

- Potassium chloride (KCl)
- Sodium acetate (NaAc)
- Hydrochloric acid (HCL)
- Acetic acid
- Buffer pH 1 (potassium chloride (M= 74,55 g/mol) solution)

A solution of 0,025 mol/L potassium chloride is produced. (1,86 KCl g/L) and adjusted to pH 1 with hydrochloric acid.

- Buffer pH 4.5 (sodium acetate (M= 82,03 g/mol) solution)

A solution of 0,4 mol/L sodium acetate is produced (32,81 NaAc g/L) and adjusted to pH 4.5 with acetic acid

Procedure

Sample preparation

See Extraction methodologies as TEAC and TPC.

- **Measurement**

The supernatant is diluted 1:10 with each buffer solution. The absorbance maximum is determined (about 500 nm, It depends on fruits variety). Each dilution is measured at the absorbance maximum and 700 nm. The spectrophotometer is zeroed with distilled water.

Notice: Dilute the sample further if absorbance is greater than 1.0 AU.

- **Calculation**
- Calculation of anthocyanins as Pg-3-glu/kg fresh weight (FW)

$$mg\,\text{Pel-3-glu}/kgFW = \frac{[(A_{\lambda\max} - A_{700})_{pH1} - (A_{\lambda\max} - A_{700})_{pH4,5}] \times MW \times F \times 1000}{\varepsilon \times d \times E}$$

A = absorbance [-]

MW = molecular weight of pelargonidin-3-glucosid = 433.2 [g/mol]

F = dilution factor [-] = 10

d = cell pathlengths [cm]

ε = molar absorbance of Pel-3-glu = 15600 $[\frac{L}{mol \times cm}]$

E = sample weight [kg/L extracting agent]

1000 = Factor for mg

Results

Anthocyanins are expressed as Pel-3-gl [mg/kg FW] fruit.

FLAVONOID CONTENT

Intention

Total flavonoid content was determined by using a colorimetric method described by Dewanto and coworkers 2002.

Material

Equipment

- Spectrophotometer
- Plastic Cuvette 1 cm (plastic)
- Volumetric flasks
- Falcon tube

Chemicals

- Sodium nitrite ($NaNO_2$) solution 5% (w/v) is produced dissolving 5 grams of sodium nitrite in 100ml distilled water.
- Alluminium Chloride ($AlCl_3*6H_2O$) solution 10% (w/v) is produced dissolving 10 grams of sodium nitrite in 100ml distilled water.
- Sodium hydroxide (NaOH) 1M solution is produced dissolving 4 g of NaOH in 100ml distilled water.
- Catechin standard is produced dissolving 0,01g of catechin in 10ml of methanol, so to obtain a batch solution with concetration 1ml/mg then dilute to obtain a standard gradient that goes from 0,0125g/L to 1g/L.

Procedure

The supernatant is diluted 1:5 with distilled water, 250 µL of sample extract or (+)-catechin standard solution was mixed with 1.25 mL of distilled water in a test tube followed by addition of 75 µL of a 5% NaNO2 solution.

After 6 min, 150 µL of a 10% AlCl3*6H2O solution was added and allowed to stand for another 5 min. Successively add 500 µL of 1 M NaOH was added. The mixture was brought to final volume of 2.5 mL with distilled water and mixed with a mechanical agitator.

The absorbance was measured immediately against the blank at 510 nm using a spectrophotometer and calculate the catechin concentration comparing the absorbance value with the calibration curve made with the standards prepared similarly with known (+)-catechin concentrations.

The results are expressed as mean (mg of Catechin Equivalents /Kg of Fresh Weight).

HPLC DETERMINATION OF VITAMIN C CONTENT.

Intention

Ascorbic acid is measured as described by Helsper *et al.* 2003.

Material

Equipment

- High Pressure Liquid Chromatography System
- mono use syringe filter sterile (size pore 0.45μm)
- C18 250 mm × 4.6 mm column
- Syringe for HPLC sample and standard injection
- Electrochemical Detector Coulochem®/Photo Diode Array (PDA) Detector
- Electronic blender
- Refrigerated centrifuge
- Ultrasonic bath

Chemicals

- Mobile phase of 10 mM phosphate buffered solution, pH 3, 5% metaphosphoric acid, isocratic gradient. Mobile phase is prepared by weighing 14,28 g (82 mmol/L) of dipotassium hydrogen phosphate (K_2HPO_4) and 2,46 g (18 mmol/L) Potassium dihydrogen phosphate (KH_2PO_4) filled up with water to 1 L and add 5grams of EDTA. Adjusted to pH 3 with hydrochloric acid.
- Solution of metaphosphoric acid 5% and 1 mM EDTA, solution of 1mM EDTA is produced diluted 0,29g of EDTA in 1L of ultrapure water for HPLC analysis, and adding 5g of metaphosphoric acid..
- Ascorbic acid Standard, diluted in ice cold solution of metaphosphoric acid 5% and 1mM EDTA. Range values that goes to 1mg/ml to 0,00125 mg/ml

Procedure

Pooled frozen strawberries are powdered with an electric blender. Vitamin C is extracted by sonication of 0.5 g of wet frozen strawberry powder in 2 mL of ice-cold water with 5% metaphosphoric acid and 1 mM EDTA during 5 minutes.

After that the strawberries are centrifuged at 2500 rpm for 10 min at 4°C, filtering with a mono use syringe filter sterile (size pore 0.45μm), and immediate analysis on an HPLC system.

The HPLC system is coupled with a C18 250 mm × 4.6 mm column. Mobile phase of 10 mM phosphate buffered solution, pH 3, with 5% of metaphosphoric acid is at isocratic gradient.

The HPLC system is coupled to an Electrochemical Detector Coulochem®. Quantification was made through a standard calibration curve prepared by running increasing standard concentrations of vitamin C (ascorbic acid) prepared similarly and measured in duplicate at the beginning and end of the analysis.

The intracultivar biological variability is assessed by separate samplings of strawberries genotypes.

Results

Results are expressed as milligrams of vitamin C (vit C) per gram of FW (SD for biological variability).

TOTAL ACIDITY

by Titrimetric Evaluation

Intention

This method is used for the determination of titratable total acid in strawberries.

The sample has to be titrated potentiometrically with 0.1 N NaOH (sodium hydroxide) to pH 8.1

Material

Equipment

- 100 mL beaker (high size)
- 2 L beaker
- 1 L volumetric flask
- balance
- hand blender
- pH - measuring instrument
- single-rod measuring cell (storage in 3 mol/L potassium chloride – solution)
- magnetic stirrer
- 50 mL burette

Chemicals

- Water (aqua dest)
- 3 mol/L potassium chloride (KCl)
- buffer solutions for calibration the pH measuring instrument at pH 4,00 and 7,00
- 0,1 n sodium hydroxide (NaOH)

- **Procedure**
  - Calibration

Calibration of the pH measuring instrument with two buffer solutions with different but exact pH-values (two-point-calibration). The buffers have to be stirred during calibration.

  - Sample Preparation

Approx. 10 g of mash strawberries are weighed exactly into a beaker and are supplemented with 10 ml of water.

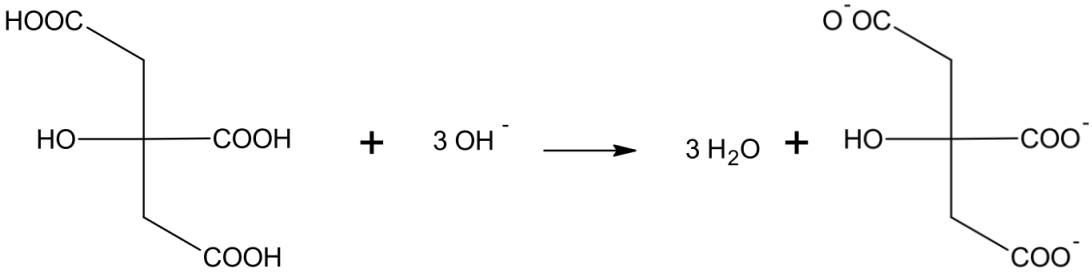
- Measuring

One aliquot of about 10 g strawberry mash, produced as described in 3.2., is given into a 100 mL beaker (high size), weighed exactly and filled up to 10 mL with distilled watrer. After immersion the single-rod measuring cell the sample has to be titrated with 0.1 N NaOH to pH 8.1 during constant stirring.

The addition of the volumetric standard solution has to be slow.

- **Calculation**

The content of total acidity will be calculated as citric acid at pH 8.1 as follows:



$$w(total\ acid) = \frac{V * c * M}{3 * E}$$

with: *w(total acid)* = content of total acid calculated as citric acid [g / kg]

V = volume of NaOH – solution [mL]

c = concentration of NaOH-solution [mol/L]

M= molecular weight of citric acid [g / mol] 192,12

E = initial weight of the mash [kg]

- **Results**

Total Acidity at pH 8,1 is expressed as [g citric acid / kg].

- **Check list**
  - Have you added 10 mL of water?
  - Is the pHmeter on?

REFERENCES

- Miller NJ, Rice-Evans C, Davis MJ (1993): A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates, clin. Sci. 84, 407-412
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999): Antioxidant activity applying an improved ABTS Radical Cation decolorization assay, Free Radical Biology & Medicine, vol. 26, No. 9/10, 1231-1237
- Giusti M. and Wrolstad R. E., Characterization and Measurement of anthocyanins by UV-visible Spectroscopy, Current Protocols in Food Analytical Chemistry, F1.2.1-F1.2.13 (2001)
- R. Matissek, F. Schnepel, G. Steiner; Lebensmittelanalytik; Springer – Verlag; Berlin Heidelberg; 1989.
- V Dewanto, X Wu, KK Adom, RH Liu. Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. J. Agric. Food Chem. 2002, 50, 3010-3014
- JPFG Helsper, *et al.* Response of selected antioxidant and pigments in tissues of *Rosa hybrida* and *Fuchsia hybrida to* supplemental UV-A exposure. Physiologia Plantarum 2003, 117:171-178

METHODOLOGIES P3

Standard Operating Procedure (SOP):

Quantification of Ascorbic Acid in Fruit

Version:		Date:	
Document id:		Filename:	
Written by:	G. Dobson	Date:	22.11.11
Approved by:		Date:	

SOP: Quantification of Ascorbic Acid in Fruit

1. INTRODUCTION

1.1 Purpose

To describe the procedure for measuring the ascorbic acid concentration in fruit. This SOP is to be used for training of new and substitute staff, for auditing purposes and for general reference.

1.2 Scope

The method can be used for fresh or frozen fruit. Either ascorbic acid only or a total of ascorbic acid and dehydroascorbate can be measured.

1.3 Responsibilities

The Laboratory Managers of the relevant laboratories are responsible for ensuring that staff are trained in the safe operation of the required apparatus and the project leader for ensuring that the staff have been trained to undertake this particular procedure.

1.4 Hazards

1.5 Overview

The fruit is homogenized and the pectin is degraded using a pectinase enzyme. The juice is recovered by centrifugation and filtration. If only the ascorbic acid concentration is to be measured, the juice is diluted with 5% metaphosphoric acid (MPA), filtered and analysed by reversed-phase HPLC using a gradient of potassium phosphate buffer (pH 2.8) and acetonitrile. If the total of ascorbic acid and dehydroascorbic acid is required, the filtered juice is diluted with phosphate buffer (pH 5.6) and treated with tris(2-carboxyethyl)phosphine hydrochloride (TCEP) to reduce the dehydroascorbic acid to ascorbic acid. Following dilution, centrifugation and filtration, the total ascorbic acid is measured by reversed phase HPLC

2. METHODS

2.1 INPUTS TO PROCESS

2.1.1 Apparatus and Reagents Required

Apparatus
2 and 4 place balances
250 mL beaker
Waring blender
High-speed centrifuge
250 ml centrifuge pots
Microcentrifuge with chilling capability (e.g. Eppendorf 5415R)
Whatman No. 1 filter paper
Measuring cylinder

1.5 mL microfuge tubes
Vortex mixer
Disposable syringe
0.2 μm filter (Whatman 25 mm GD/X)
10 mL volumetric flask
HPLC system consisting of binary (or tertiary, quaternary) pump, autosampler, PDA detector, column heater and data system.
Synergi 4 μ Hydro-RP 80A HPLC column (250 mm x 4.6 mm; 4 μm) (Phenomenex)
Synergi 4 μ Hydro-RP 80A guard column (5 mm x 4.6 mm; 4 μm) (Phenomenex)

Chemicals
Pectinex 5X (Novozymes A/S)
Metaphosphoric acid (60% + 40% sodium phosphate stabiliser)
Sodium dihydrogen phosphate, monohydrate
Disodium hydrogen phosphate heptahydrate
tris(2-carboxyethyl)phosphine hydrochloride (TCEP)
Ascorbic acid (>99% purity)
Acetonitrile
Formic acid

Preparation of solutions and buffers
5% MPA containing 5 mM TCEP. 2.5 g MPA in 25 mL water in volumetric flask and 35.83 mg TCEP, sonicate to dissolve, aliquot 1 mL into microfuge tubes and freeze -80°C).
100mM sodium phosphate buffer (pH 5.6).1.309 g monosodium phosphate, monohydrate and 0.138 g disodium phosphate heptahydrate in 100 mL water.

2.2. PROCEDURES

2.2.1 Sample preparation

2.2.1.1 Remove frozen fruit from storage (-20°C freezer) and weigh 150 g into a beaker. Allow to thaw at room temperature for approximately 4 h.
2.2.1.2 Add 150 μl Pectinex 5X to the fruit and homogenize using a Waring blender for 60 s.
2.2.1.3 Cover beaker with aluminium foil and leave at room temperature overnight.
2.2.1.4 Transfer sample to centrifuge pot and centrifuge at 5000 x g for 20 min at 1°C for 20 min.
2.2.1.5 Filter sample into measuring cylinder using Whatman No. 1 filter paper. Note the volume of juice. Decant juice into 1.5 mL microfuge tubes and freeze at -20°C. Prior to analysis thaw tube at room temperature.
2.2.1.6 For measurement of ascorbic acid only, add 20 μl of filtered sample to 980 μl 5% (wt/vol) metaphosphoric acid (MPA) in a 1.5 ml microfuge tube, vortex and centrifuge 16000 x g for 5 min at 1°C.
2.2.1.7 Using a disposable syringe, put sample through 0.2 μm filter into microfuge tube. Transfer 200 μl of filtered sample to an autosampler vial. The sample is now ready for HPLC analysis.
2.2.1.8 For measurement of total of ascorbic and dehydroascorbic acid, 50 μl juice prepared in 2.2.6 was diluted with 450 μl 100 mM phosphate buffer (pH 5.6) in a 1.5 ml microfuge tube and, after vortexing, 100 μl was transferred to another

microfuge tube. Add 2 μl 5% MPA containing 5 mM TCEP and leave for 18 h at room temperature.

2.2.1.9 Add 100 μl 10% MPA, centrifuge 16000 x g for 5 min at 1°C and filter as in 2.2.8. The sample is now ready for HPLC analysis.

2.2.1.10 Standard solutions are prepared by first preparing a 10 mg/mL solution of ascorbic acid; accurately weigh approximately 100 mg ascorbic acid into a 10 mL volumetric flask, make up to the mark with 5% MPA containing 5 mM TCEP and shake until dissolved. Prepare 1 mg/mL solution by adding 100 μl to 900 μl 5% MPA containing 5 mM TCEP in a microfuge tube and vortexing. In another 10 mL volumetric flask, prepare 0.1 mg/mL solution by adding 1.0 mL of the 1mg/mL solution and make up to the mark with 5% MPA containing 5 mM TCEP. In microfuge tubes,10, 20, 40, 60, 80 and 100 μg/mL solutions are prepared by adding 100, 200, 400, 600, 800 and 1000 μl of the 0.1 mg/mL solution to 900, 800, 600, 400, 200 and 0 μl 5% MPA containing 5 mM TCEP, respectively. Transfer 200 μl of each standard to an autosampler vial.

2,2,2 HPLC analysis

2.2.1 The samples and standards are injected in duplicate via an autosampler on to a reversed-phase HPLC column linked to a PDA detector. A pump capable of binary gradients is used. Parameters for the autosampler, mobile phase and PDA are as follows:-

Injection volume: 20 μl.

Autosampler temperature: 5°C

Column type:Synergi 4 μ Hydro-RP 80A HPLC column (250 mm x 4.6 mm; 4 μm)
Guard column:Synergi 4 μ Hydro-RP 80A guard column (5 mm x 4.6 mm; 4 μm)

Column temperature: 30°C.

Mobile phase: Solvent A – 0.1% aqueous formic acid
Solvent B – acetonitrile

Flow rate: 2.5 mL min^{-1}

Gradient :

Time (min)	A%	B%
0	100	0
0.50	100	0
0.75	20	80
1.25	20	80
1.50	100	0
3.00	100	0

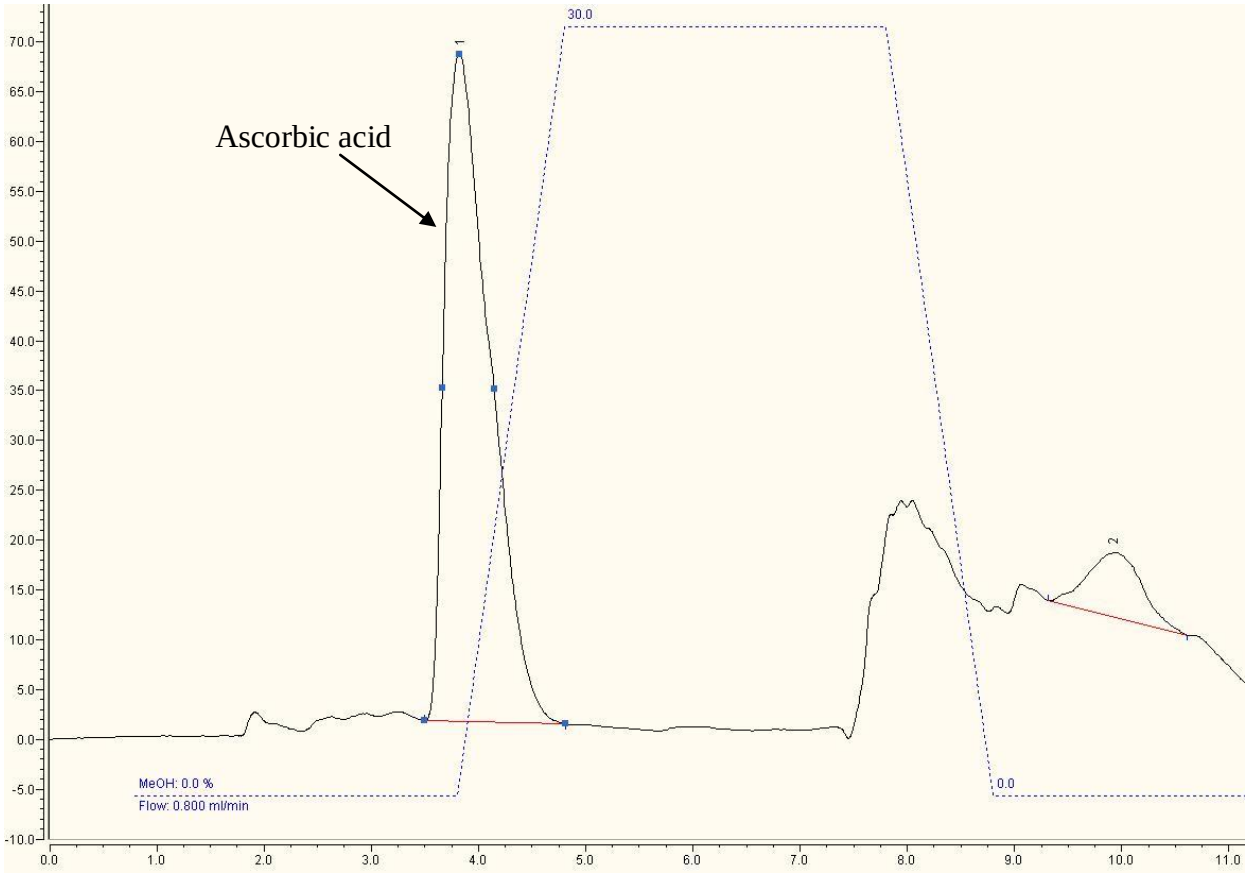
PDA range: 200-600 nm.

PDA channel: 245 nm

2.2.3 Quantification

The areas at 245 nm of the ascorbic acid peaks in the calibration standards are plotted against the concentration (μg/mL) and the equation of the best-fit line determined in Excel. The identity of the ascorbic acid peak in the samples is determined by comparison of retention time to that of the standards, and the areas are measured. Concentrations (μg/mL) in the samples are determined using the calibration equation. The concentrations (μg/mL) in the juice extracts are calculated by multiplying by the dilution factors – x50 for ascorbic acid determination and x20 for total of ascorbic acid and dehydroascorbic acid determination. The concentration (μg/g) in the original fruit can then be determined by multiplying by the total volume (mL) of juice and dividing by the mass of fruit (g).

Appendix HPLC profile of ascorbic acid in blackcurrant



Standard Operating Procedure (SOP):

Quantification of Sugars in Fruit

Version:		Date:	
Document id:		Filename:	
Written by:	G. Dobson	Date:	22.11.11
Approved by:		Date:	

SOP: Quantification of Sugars in Fruit

1. INTRODUCTION

1.1 Purpose

To describe the procedure for measuring the concentration of individual and total sugars in fruit. This SOP is to be used for training of new and substitute staff, for auditing purposes and for general reference.

1.2 Scope

The method can be used for fresh or frozen fruit. The method measures glucose, fructose and sucrose concentrations.

1.3 Responsibilities

The Laboratory Managers of the relevant laboratories are responsible for ensuring that staff are trained in the safe operation of the required apparatus and the project leader for ensuring that the staff have been trained to undertake this particular procedure.

1.4 Hazards

1.5 Overview

The fruit is homogenized and the pectin is degraded using a pectinase enzyme. The juice is recovered by centrifugation and filtration. After suitable dilution of the juice concentration of individual sugars are analysed on an anion exchange HPLC column and an isocratic mobile phase of 200 mM sodium hydroxide. Calibration standards of the relevant sugars are run and the concentration of individual or total sugars in the juices are thereby determined.

2. METHODS

2.1 INPUTS TO PROCESS

2.1.1 Apparatus and Reagents Required

Apparatus
2 and 4 place balances
250 mL beaker
Waring blender
High-speed centrifuge
250 ml centrifuge pots
Microcentrifuge with chilling capability (e.g. Eppendorf 5415R)
Whatman No. 1 filter paper
Measuring cylinder
1.5 mL microfuge tubes
Vortex mixer
0.2 μm filter (Whatman 25 mm GD/X)

10 mL volumetric flask
Dionex high-performance anion exchange chromatographic (HPAEC) consisting of pump, autosampler, column heater, pulsed amperometer detector and data system.
Dionex Carbopac PA-100 (250 mm x 4 mm) column.

Chemicals
Pectinex 5X (Novozymes A/S)
Sodium hydroxide
Glucose
Fructose
Sucrose

Preparation of mobile phase
200 mM sodium hydroxide prepared by dissolving 8 g of sodium hydroxide in a litre of distilled water.

2.2. PROCEDURES

2.2.1 Juice extraction

2.2.1.1 Remove frozen fruit from storage (-20°C freezer) and weigh 150 g into a beaker. Allow to thaw at room temperature for approximately 4 h.
2.2.1.11 Add 150 μl Pectinex 5X to the fruit and homogenize using a Waring blender for 60 s.
2.2.1.12 Cover beaker with aluminium foil and leave at room temperature overnight.
2.2.1.13 Transfer sample to centrifuge pot and centrifuge at 5000 x g for 20 min at 1°C for 20 min.
2.2.1.14 Filter sample into measuring cylinder using Whatman No. 1 filter paper. Note the volume of juice. Decant juice into 1.5 mL microfuge tubes and freeze at -20°C. Prior to analysis thaw tube at room temperature.

2.2.2 Sample preparation

2.2.2.1 Add 100 μl of filtered sample to 900 μl distilled water in a 1.5 ml microfuge tube and vortex. Add 100 μl of this dilution to 900 μl distilled water in another microfuge tube. Repeat similar 10 fold dilutions twice more and then add 200 μl of the final dilution to 800 μl distilled water in another microfuge tube so that a final dilution of 1 in 5000 of the juice is achieved. Incubate final dilution at 100°C in an oven for 5 min.
2.2.2.2. Centrifuge 16000 x g for 5 min at 1°C and transfer 200 μl of sample to HPLC vials ready for HPLC analysis.
2.2.2.3 Standard solutions are prepared of individual sugars (normally fructose, glucose and sucrose) by first preparing a solution of each sugar at 2 mg/mL; accurately weigh approximately 20 mg sugar into a 10 mL volumetric flask, make up to the mark with distilled water and shake until dissolved. Prepare a mixture of each sugar at 200 μg /mL solution by adding 1.0 mL of each solution to another 10 mL volumetric flask, make up to the mark with distilled water and shake. In another 10 mL volumetric flask prepare 20 μg /mL solution by adding 1.0 mL of the 200 μg /mL solution and making up to the mark with distilled water. In microfuge tubes, 2, 4, 8, 12, 16 and 20 μg/mL solutions are prepared by adding 100, 200, 400, 600,

800 and 1000 μl of the 200 μg /mL solution to 900, 800, 600, 400, 200 and 0 μl distilled water, respectively. Transfer 200 μl of each standard to an autosampler vial.

2,2.3 HPLC analysis

The samples and standards are injected in duplicate via an autosampler on to a anion exchange HPLC column linked to a pulsed amperometer detector. A pump capable of isocratic delivery is used. Parameters for the autosampler, mobile phase and detector are as follows:-

Injection volume: 25 μl.

Autosampler temperature: 5°C

Column type: Dionex Carbopac PA-100 (250 mm x 4 mm)

Column temperature: 30°C.

Mobile phase: 200 mM sodium hydroxide (isocratic)

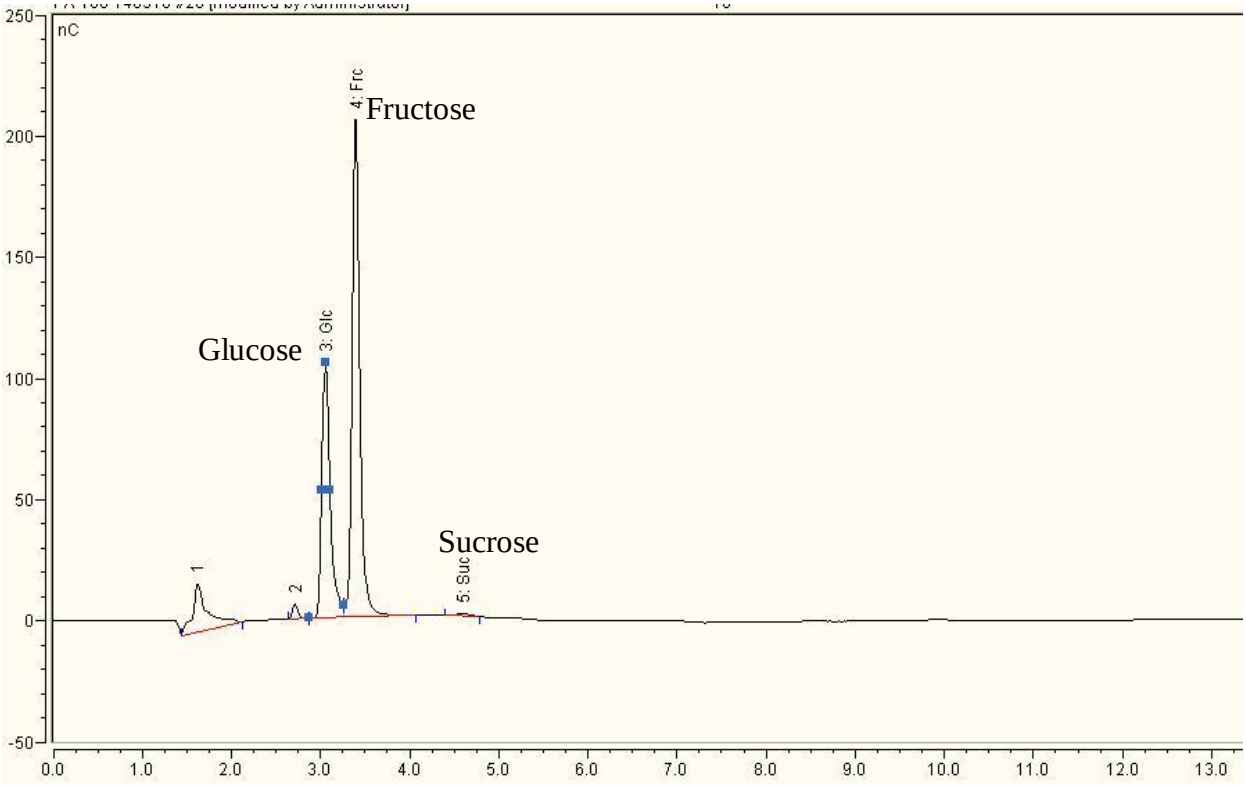
Flow rate: 1.0 mL min^{-1}

Detector: Pulsed amperometer in standard quad waveform mode

2.2.4 Quantification

The areas of the sugar peaks in the calibration standards are plotted against the concentration (μg/mL) and the equation of the best-fit line determined in Excel. The identities of the sugar peaks in the samples are determined by comparison of retention times to those of the standards, and the areas are measured. Concentrations (μg/mL) in the samples are determined using the calibration equation. Considering that the juices were diluted 1000 fold, this value is the concentration in mg/mL in the undiluted juice extracts. The concentration (mg/g) in the original fruit can then be determined by multiplying by the total volume (mL) of juice and dividing by the mass of fruit (g). Results are expressed as concentration of individual or total sugars.

Appendix HPLC profile of sugars in blackcurrant


nC
4: Frc
Fructose
3: Glc
Glucose
1
2
5: Suc
Sucrose
250
200
150
100
50
0
-50
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0

Time (min)

Standard Operating Procedure (SOP):

Quantification of Organic Acids in Fruit

Version:		Date:	
Document id:		Filename:	
Written by:	G. Dobson	Date:	22.11.11
Approved by:		Date:	

SOP: Quantification of Organic Acids in Fruit

1. INTRODUCTION

1.1 Purpose

To describe the procedure for measuring the concentration of individual and total organic acids in fruit. This SOP is to be used for training of new and substitute staff, for auditing purposes and for general reference.

1.2 Scope

The method can be used for fresh or frozen fruit. Often only citrate, malate and oxalate are measured but the method can be extended to include other organic acids such as fumarate, isocitrate, succinate and tartarate.

1.3 Responsibilities

The Laboratory Managers of the relevant laboratories are responsible for ensuring that staff are trained in the safe operation of the required apparatus and the project leader for ensuring that the staff have been trained to undertake this particular procedure.

1.4 Hazards

1.5 Overview

The fruit is homogenized and the pectin is degraded using a pectinase enzyme. The juice is recovered by centrifugation and filtration. After suitable dilution of the juice, concentration of individual organic acids are analysed on an anion exchange HPLC column and with a gradient of 10% methanol and 100 mM sodium hydroxide in 10% methanol. Calibration standards of the relevant organic acids are run and the concentration of individual or total organic acids in the juices are thereby determined.

2. METHODS

2.1 INPUTS TO PROCESS

2.1.1 Apparatus and Reagents Required

Apparatus
2 and 4 place balances
250 mL beaker
Waring blender
High-speed centrifuge
250 ml centrifuge pots
Microcentrifuge with chilling capability (e.g. Eppendorf 5415R)
Whatman No. 1 filter paper
Measuring cylinder
1.5 mL microfuge tubes
Vortex mixer

0.2 μm filter (Whatman 25 mm GD/X)
10 mL volumetric flask
Dionex high-performance anion exchange chromatographic (HPAEC) consisting of pump, autosampler, column heater, electrochemical detector and data system.
Dionex IonPac AS11-HC (250 mm x 4 mm) column.
Guard column of Dionex IonPac AS11-HC (50 mm x 4 mm) column.

Chemicals
Pectinex 5X (Novozymes A/S)
Sodium hydroxide
Methanol (HPLC grade)
Ultrapure water
Citric acid
Malic acid
Oxalic acid

Preparation of mobile phase
100 mM sodium hydroxide in 10% methanol prepared by dissolving 4 g of sodium hydroxide in 900 mL ultrapure water and adding 100 mL methanol

2.2. PROCEDURES

2.2.1 Juice extraction

2.2.1.1 Remove frozen fruit from storage (-20°C freezer) and weigh 150 g into a beaker. Allow to thaw at room temperature for approximately 4 h.
2.2.1.15 Add 150 μl Pectinex 5X to the fruit and homogenize using a Waring blender for 60 s.
2.2.1.16 Cover beaker with aluminium foil and leave at room temperature overnight.
2.2.1.17 Transfer sample to centrifuge pot and centrifuge at 5000 x g for 20 min at 1°C for 20 min.
2.2.1.18 Filter sample into measuring cylinder using Whatman No. 1 filter paper. Note the volume of juice. Decant juice into 1.5 mL microfuge tubes and freeze at -20°C. Prior to analysis thaw tube at room temperature.

2.2.2 Sample preparation

2.2.2.1 Make a 50 fold dilution of the filtered sample by adding 30 μl to 1200 μl ultrapure water in a 2 ml microfuge tube and vortex.
2.2.2.2. Centrifuge 16000 x g for 5 min at 1°C and transfer 200 μl of sample to HPLC vials ready for HPLC analysis.
2.2.2.4 Standard solutions are prepared of individual organic acids (normally citric acid, malic acid and oxalic acid) by first preparing a solution of each organic acid at 5 mg/mL; accurately weigh approximately 50 mg organic acid into a 10 mL volumetric flask, make up to the mark with ultrapure water and shake until

dissolved. Prepare a mixture of each organic acid at 1 mg /mL solution by adding 2.0 mL of each solution to another 10 mL volumetric flask, make up to the mark with ultrapure water and shake. In microfuge tubes, 20, 50, 100, 400, 700 and 1000 μg/mL solutions are prepared by adding 20, 50, 100, 400, 800 and 1000 μl of the 1 mg/mL solution to 908, 950, 900, 600, 200 and 0 μl ultrapure water, respectively. Transfer 200 μl of each standard to an autosampler vial.

2,2.3 HPLC analysis

The samples and standards are injected in duplicate via an autosampler on to an anion exchange HPLC column linked to an electrochemical detector. A pump capable of binary gradients is used. Parameters for the autosampler, mobile phase and electrochemical detector are as follows:-

Injection volume: 25 μl.

Autosampler temperature: 5°C

Column type: Dionex IonPac AS11-HC (250 mm x 4 mm)

Guard column: Dionex IonPac AS11-HC (50 mm x 4 mm)

Column temperature: 30°C.

Mobile phase: Solvent A – 10% aqueous methanol
Solvent B – 100 mM NaOH in 10% aqueous methanol

Flow rate: 1.5 mL min^{-1}

Gradient :

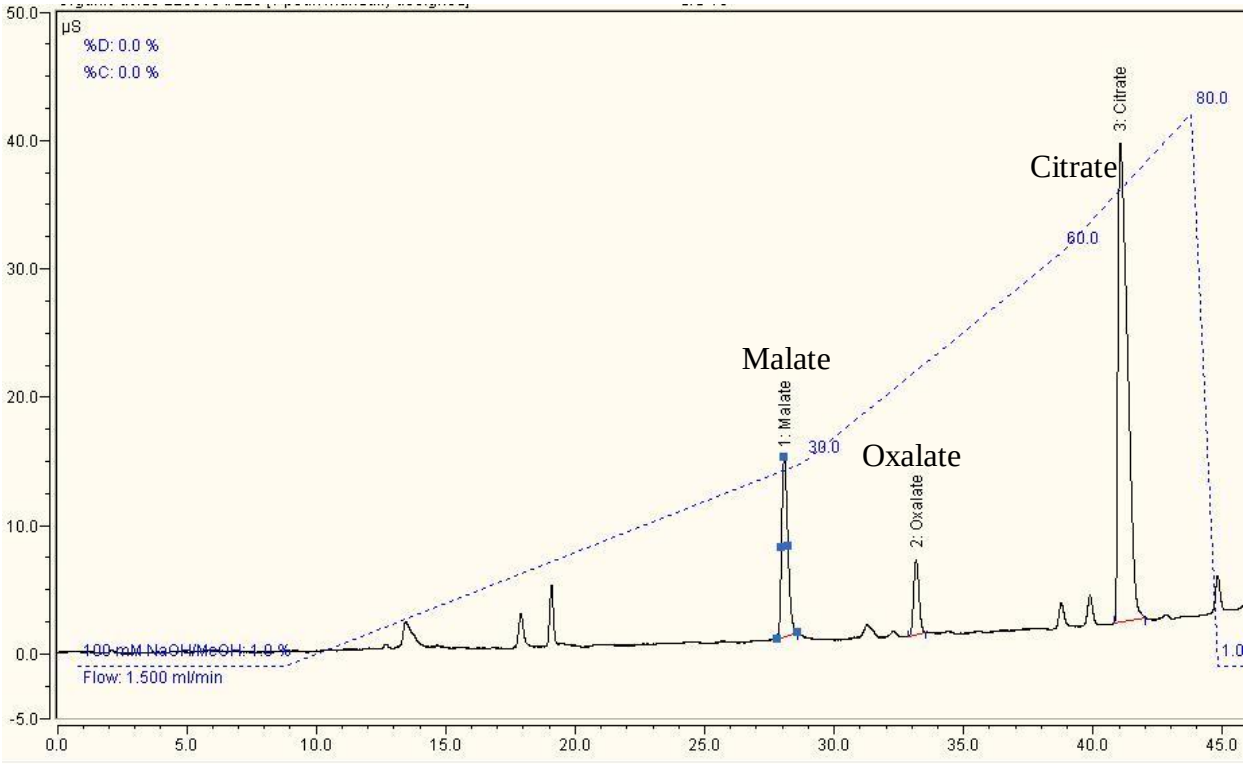
Time (min)	A%	B%
0	99	1
8	99	1
28	70	30
38	40	60
43	20	80
44	99	1
50	99	1

Detector: Electrochemical detector. The detector was preceded by a 4 mm ASRS 300 anion self-generating suppressor used in the external mode to suppress the background conductivity of the mobile phase. Ion suppression was undertaken at 240 mA with ultrapure water at 2 mL min^{-1}.

2.2.4 Quantification

The areas of the organic acid peaks in the calibration standards are plotted against the concentration (μg/mL) and the equation of the best-fit line determined in Excel. The identities of the organic acid peaks in the samples are determined by comparison of retention times to those of the standards, and the areas are measured. Concentrations (μg/mL) in the samples are determined using the calibration equation. Considering that the juices were diluted 50 fold, multiply this value by 0.05 to give the concentration in mg/mL in the undiluted juice extract. The concentration (mg/g) in the original fruit can then be determined by multiplying by the total volume (mL) of juice and dividing by the mass of fruit (g). Results are expressed as concentration of individual or total organic acids.

Appendix HPLC profile of organic acids in blackcurrant



Standard Operating Procedure (SOP):

Analysis of Polyphenolics in Raspberry, Blackberry, Blueberry, Black Currant and Strawberry by Ultra High Pressure Liquid Chromatography-Photodiode Array-Mass Spectrometry (UPLC-PDA-MS)

Version:		Date:	
Document id:		Filename:	
Written by:	G. Dobson	Date:	03.11.11
Approved by:		Date:	

SOP: Analysis of Polyphenolics in Raspberry, Blackberry, Blueberry, Black Currant and Strawberry by Ultra High Pressure Liquid Chromatography-Photodiode Array-Mass Spectrometry (UPLC-PDA-MS)

1. INTRODUCTION

1.1 Purpose

To describe the procedure for extracting the polyphenolic compounds from raspberry, blackberry, blueberry, black currant or strawberry and subsequent analysis by ultra High Pressure liquid chromatography-mass spectrometry. This SOP is to be used for training of new and substitute staff, for auditing purposes and for general reference.

1.2 Scope

The method can be used for fresh, frozen or freeze-dried raspberries.

1.3 Responsibilities

The Laboratory Managers of the relevant laboratories are responsible for ensuring that staff are trained in the safe operation of the required apparatus and the project leader for ensuring that the staff have been trained to undertake this particular procedure.

1.4 Hazards

1.5 Overview

The method is for extracting polyphenolics from raspberry, blackberry, blueberry, black currant or strawberry and subsequent analysis by UPLC-PDA-MS. Extraction is carried out by homogenizing fresh or frozen raspberries with 0.5% formic acid in acetonitrile followed by centrifugation. The solvent is removed by Speed Vac and then freeze-dried to remove any remaining water. The sample is re-dissolved in 0.5% formic acid in 5% aqueous acetonitrile and is analysed by reversed phase UPLC-PDA-MS using a mobile phase of 0.1% formic acid in water with increasing proportions of acetonitrile.

2. METHODS

2.1 INPUTS TO PROCESS

2.1.1 Apparatus and Reagents Required

Apparatus
Balance (3 place or better)
Microcentrifuge (Eppendorf 5415D or equivalent)
Borosilicate glass mortars (homogenisers), 5 mL capacity
1.5ml and 2ml microfuge tubes

8ml glass vials
96 well microfuge racks
Scalpel
Freeze-drier

Speed vac SPD131DA (Savant) or equivalent, with refridgerated vapour trap and pump
Eppendorf P1000 pipette or equivalent
Handystep electronic pipette (Brand)
Single StEP^{TM} filter vial 0.45 μm PTFE (Thomson)
Thermo LCQ fleet ion trap mass spectrometer with Accela 600 pump, autosampler and PDA detector or equivalent
Hypersil Gold (50 mm x 2.1 mm; 1.9 μm reversed-phase UPLC column (Thermo)

Chemicals
Acetonitrile
Formic acid
Double distilled water

2.2 PROCEDURES

2.2.1 Extraction of polyphenolics

2.2.1.1 Using scalpel cut 3 fresh or frozen berries in half (quarters for strawberry) and weigh in a weighing boat. If it is not possible to obtain whole fruit, accurately weigh out approximately 3 g.
2.2.1.2 Transfer fruit to homogeniser and add volume of 0.5% formic acid in acetonitrile equal to the weight of berries. Break up with spatula, allow frozen berries to thaw, crush with pestle and transfer to 2 mL microfuge tubes.
2.2.1.3 Centrifuge samples at 13200 rpm for 5 min and transfer supernatants to a 2 mL micorfuge tube using automatic pipette.
2.2.1.4 Centrifuge samples at 13200 rpm for 3 min and combine supernatants in an 8 mL vial.
2.2.1.5 Mix sample and transfer 500 ul aliquots to 1.5 mL microfuge tubes using Handystep.
2.2.1.6 Store samples in -20°C freezer until ready to be taken to dryness.
2.2.1.7 Thaw samples in cold room (-4°C) and leave on Speed Vac at 45°C for 3 h. Freeze samples in -20°C freezer.
2.2.1.8 Freeze-dry samples overnight using side arm of freeze-drier and keep in -18°C freezer.

2.2.2 Analysis of polyphenolics by UPLC-PDA-MS

2.2.2.1 Prior to use, dissolve sample in 0.5% formic acid in water/acetonitrile (95:5, vol/vol) at same concentration as that (usually 500 ul) prior to freeze-drying, vortex to disrupt pellet and centrifuge at 13200 rpm for 3 min. Transfer 0.4 mL sample to filter vial and push filter into vial.
2.2.2.2 The sample is injected via an autosampler on to a UPLC column linked to a PDA system (Thermo Accela) and then an electrospray ionization ion trap mass spectrometer (Thermo LCQ Fleet). Details of the parameters for the autosampler, mobile phase, PDA and mass spectrometer are given in Appendix. The important parameters are as follows:-

Injection volume: 8 μl.

Column type: Hypersil Gold (50 mm x 2.1 mm; 1.9 μm) (Thermo)

Autosampler temperature: 6°C

Column temperature: 30°C.

Mobile phase: Solvent A – 0.1% (vol/vol) aqueous formic acid
Solvent B – 0.1% (vol/vol) formic acid in acetonitrile/water (50:50, vol/vol).

Flow rate: 450 μl min^{-1}

Gradient :

Time (min)	A%	B%
0	97	3
3	85	15
7	75	25
10	50	50
13	50	50
14	100	0
16	100	0

PDA range: 200-600 nm.

PDA channels: A. 280 nm (general polyphenols).
B. 365 nm (flavonols).
C 520 nm (anthocyanins).

Mass range: *m/z* 100-2000.

Source parameters: Capillary temperature 300°C, sheath gas and auxillary gas 35 and 5 units and spray voltage 3.8 kV

Scan events: Alternative full scan MS and MS/MS (data-dependant scan).

The sample is run twice – in positive ion and negative ion modes.

2.3 POLYPHENOL COMPOSITION

2.3.1 Identification

Identification of polyphenols is based on PDA characteristics and mass spectra including MS and MS/MS data.

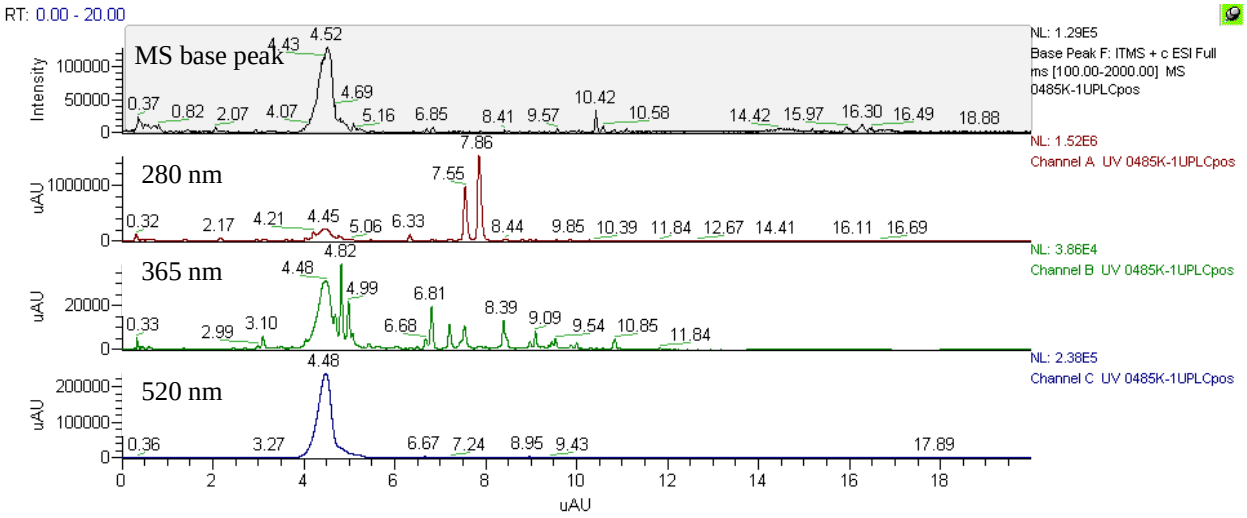
2.3.1.1 Raspberry polyphenols

Raspberry contains mainly anthocyanins (analysed in positive ion mode) and ellagitannins (negative ion mode) with flavonols (negative and sometimes positive ion modes) as minor compounds.
The following table gives a list of polyphenols found in raspberry and expected masses.

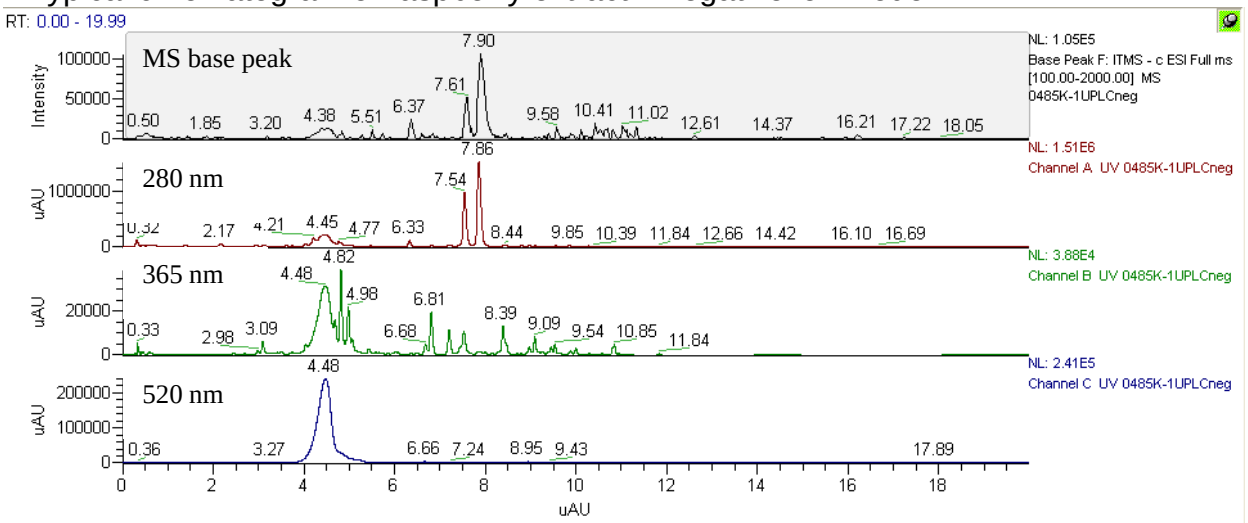
Compound	MS	MS2
Anthocyanins	**$[M+H]^+$**	
Cyanidin 3-sophoroside	611	287
Cyanidin 3,5-diglucoside	611	449, 287
Cyanidin 3-(2'-glucosyl)rutinoside	757	287
Cyanidin 3-sophoroside-5-rhamnoside	757	611, 433, 287
Cyanidin 3-sambubioside	581	287
Cyanidin 3-(2'-xylosyl)rutinoside	727	287
Cyanidin 3-glucoside	449	287
Cyanidin 3-sambubioside-5-rhamnoside	727	581, 433, 287
Cyanidin 3-rutinoside	595	449, 287
Pelargonidin 3-sophoroside	595	271
Pelargonidin 3-(2'-glucosyl)rutinoside	741	271
Pelargonidin 3-sambubioside	565	271
Pelargonidin 3-glucoside	433	271
Pelardonidin 3-rutinoside	579	433, 271
Flavonols	**$[M-H]^-$**	
Quercetin galactosylrhamnoside	609	301
Quercetin (2'-glucosyl)rutinoside	771	301
Quercetin galactoside	463	301
Quercetin glucoside	463	301
Quercetin glucuronide	477	301
Quercetin diglucoside	625	301
Quercetin rutinoside	609	301
Kaempferol glucuronide	461	285
Methylquercetin glucuronide	491	315
Ellagitannins/ellagic acid derivatives	**$[M-H]^-$**	
Sanguiin-H6	$[1869]^{-1}$ $[935]^{-1}$	1697, 1567, 1407,897, 783, 633, 301
Lambertianin C	$[1401]^{-2}$	2019, 1869, 1567, 1402, 935, 897, 633, 301
Ellagic acid pentoside 1	433	301
Ellagic acid pentoside 2	433	301
Ellagic acid acetyl arabinoside	475	301
Ellagic acid 4-acetyl xyloside	475	301
Ellagic acid	301	
Ellagic acid rhamnoside	447	301
Ellagic acid pentose conjugate	433	
Methyl ellagic acid pentose conjugate	477	

The following chromatograms show typical raspberry (cultivar 0485K-1) profiles and identification of some major components.

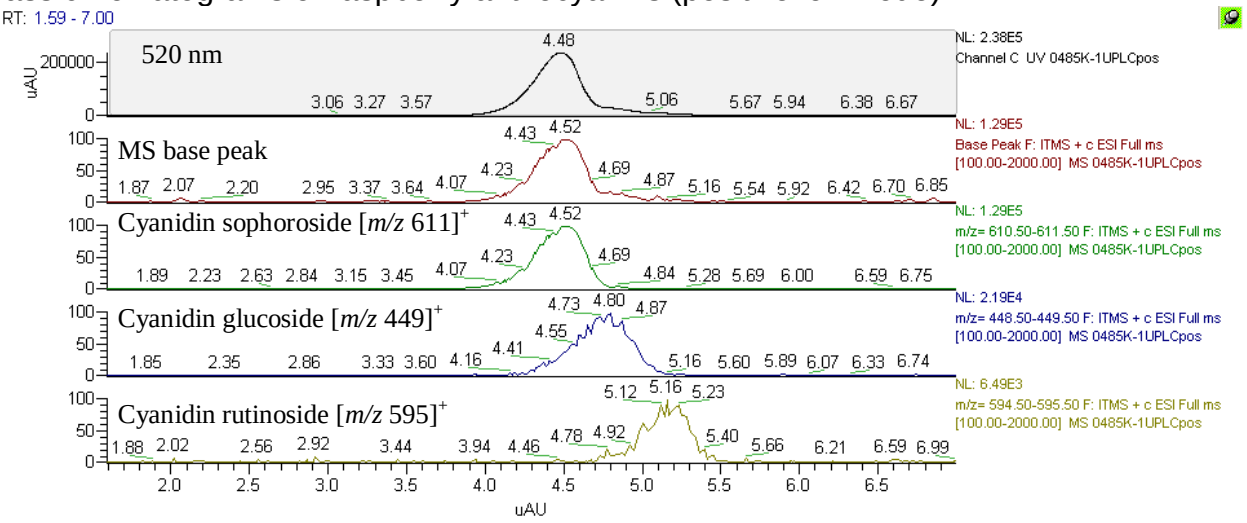
Typical chromatogram of raspberry extract – positive ion mode



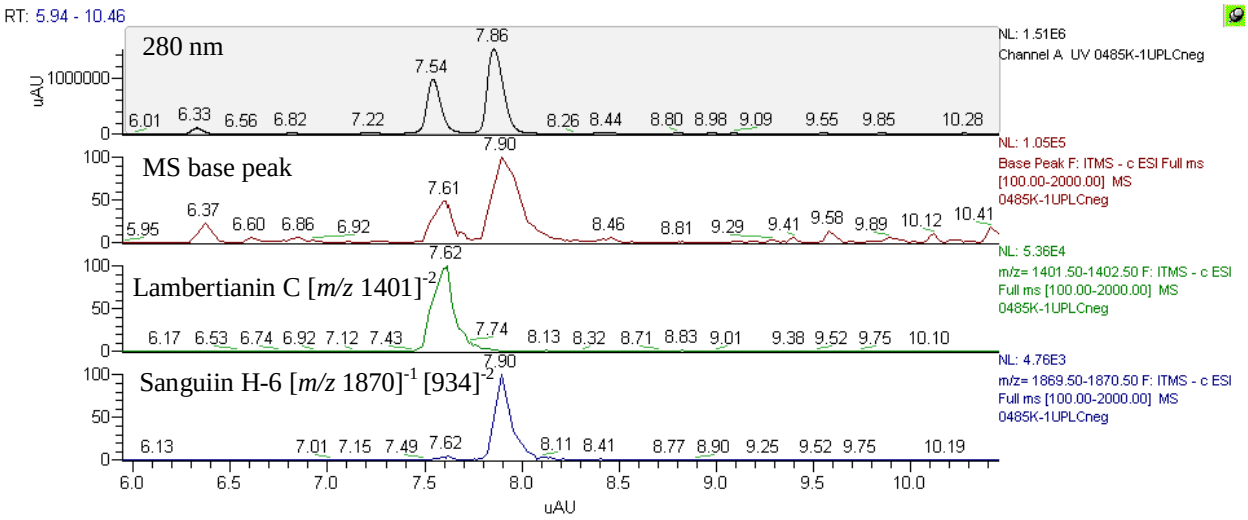
Typical chromatogram of raspberry extract – negative ion mode



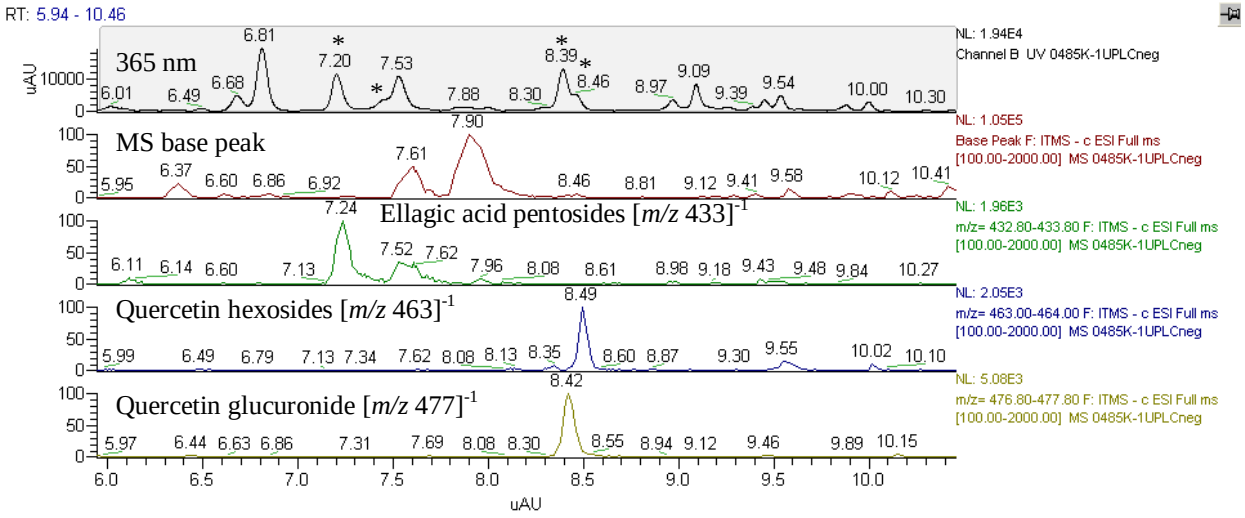
Mass chromatograms of raspberry anthocyanins (positive ion mode)



Mass chromatograms of raspberry ellagitannins (negative ion mode)



Mass chromatograms of raspberry flavonols (negative ion mode)



2.3.1.2 Blackberry polyphenols

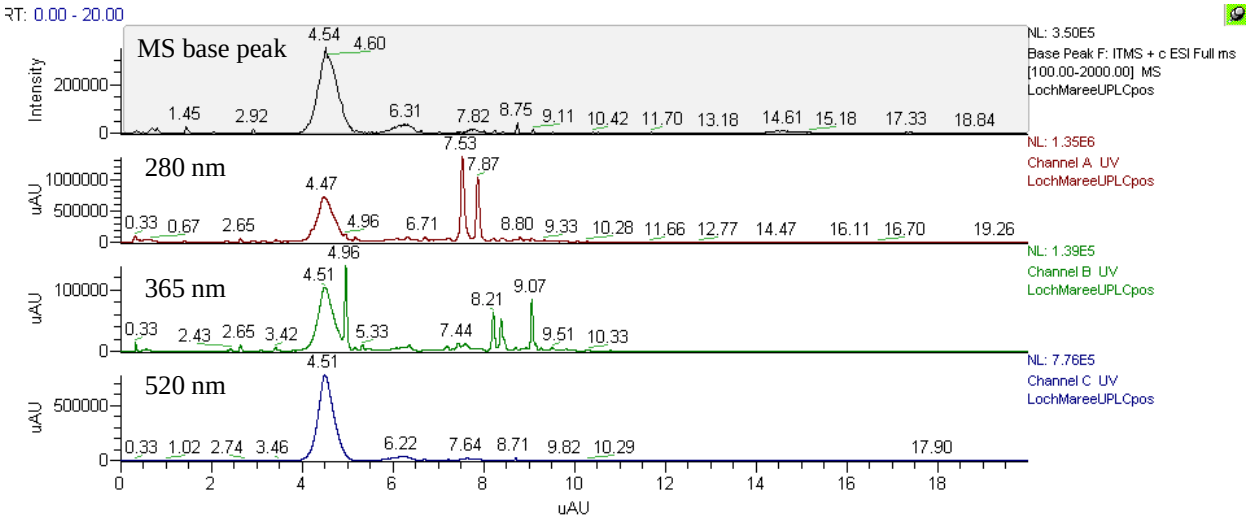
Blackberry contains mainly anthocyanins (analysed in positive ion mode) and ellagitannins (negative ion mode) with flavonols (negative and sometimes positive ion modes) as minor compounds.

The following table gives a list of polyphenols found in blackberry and expected masses.

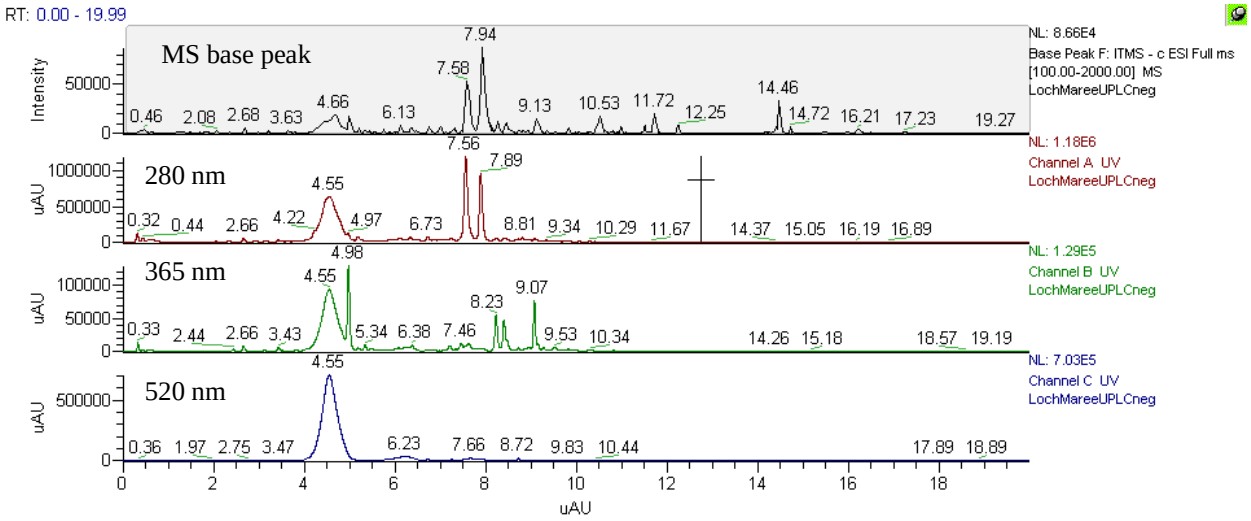
Compound	MS	MS2
Anthocyanins	**$[M+H]^+$**	
Cyanidin 3-glucoside	449	287
Cyanidin 3-arabinoside	419	287
Cyanidin 3-rutinoside	595	449, 287
Pelargonidin 3-glucoside	433	271
Cyanidin 3-(3'-malonyl)glucoside	535	287
Peonidin 3-glucoside	463	301
Cyanidin 3-xyloside	419	287
Cyanidin 3-(6'-malonyl)glucoside	535	449, 287
Cyanidin 3-dioxalylglucoside	593	287
Cyanidin 3-[6''-(3-hydroxy-3-methylglutaroyl) galactoside	593	287
Flavonols	**$[M-H]^-$**	
Quercetin 3-rutinoside	609	463, 301
Quercetin 3-galactoside	463	301
Quercetin 3-methoxyhexoside	493	463, 301
Quercetin 3-glucoside	463	301
Quercetin 3-pentoside 1	433	301
Quercetin 3-pentoside 2	433	301
Quercetin glucuronide	477	301
Quercetin 3-[6''-(3-hydroxy-3-methylglutaroyl) galactoside	607	463, 301
Quercetin acetylhexoside	505	301
Quercetin 3-glucosylpentoside	595	433, 301
Quercetin 3-oxalylpentoside	505	433, 301
Quercetin	301	
Kaempferol hexoside	447	285
Kaempferol 3-[6''-(3-hydroxy-3-methylglutaroyl) galactoside	591	285
Ellagitannins/ellagic acid derivatives	**$[M-H]^-$**	
Sanguiin H-2	1103	
Sanguiin H-6	1869	301
Sanguiin-H6	$[1869]^{-1}$ $[935]^{-1}$	1697, 1567, 1407,897, 783, 633, 301
Lambertianin C	$[1401]^{-2}$	2019, 1869, 1567, 1402, 935, 897, 633, 301
Ellagic acid	301	
Methyl ellagic acid glucuronide	521	315
Methyl ellagic acid pentose	477	315

The following chromatograms show typical blackberry (cultivar Loch Maree) profiles and identification of some major components.

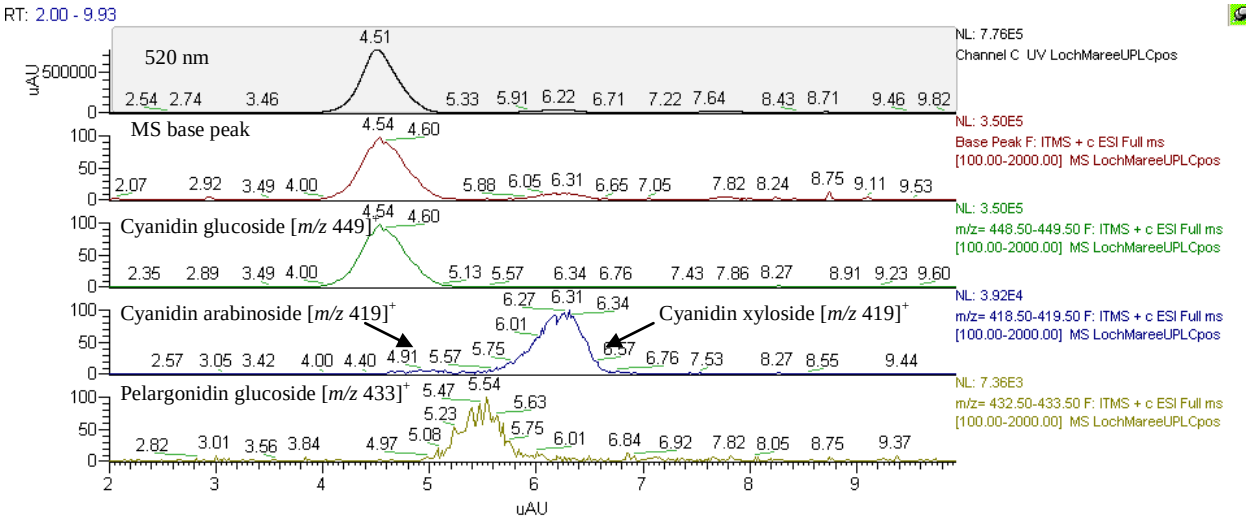
Typical chromatogram of blackberry extract – positive ion mode

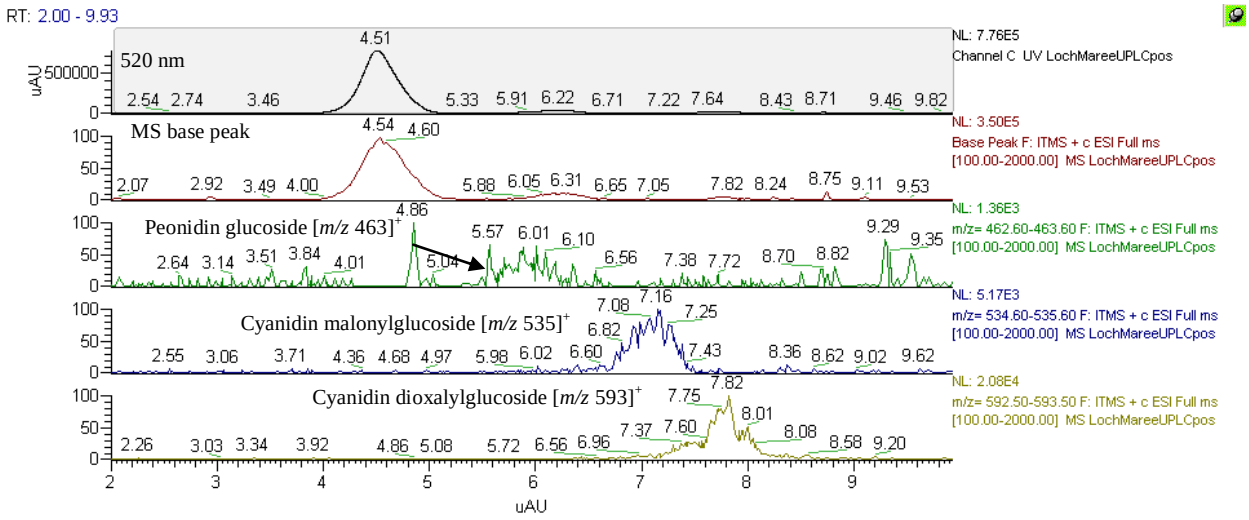


Typical chromatogram of blackberry extract – negative ion mode

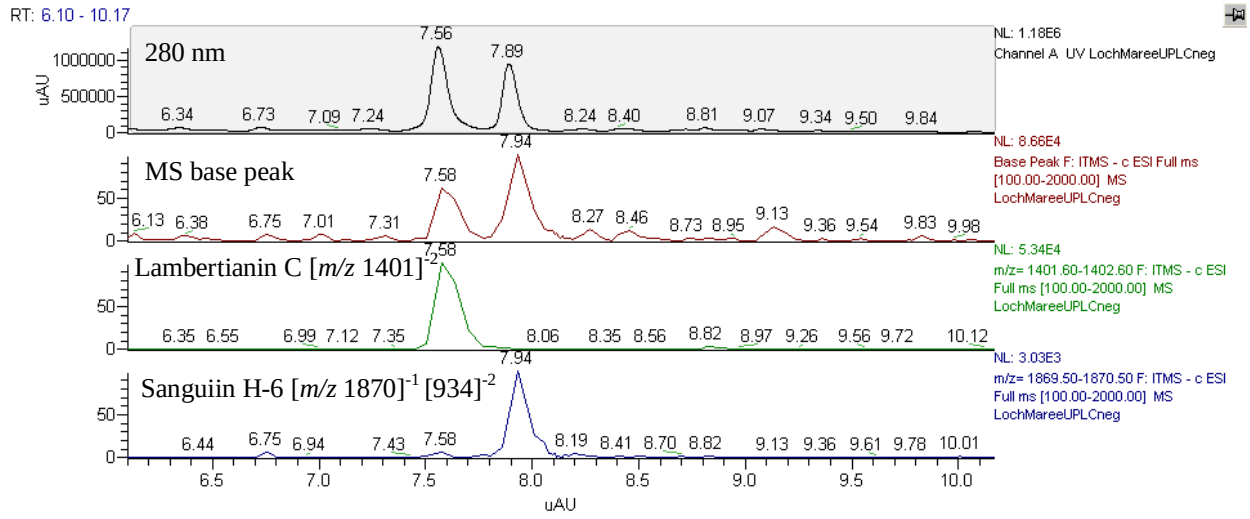


Mass chromatograms of blackberry anthocyanins (positive ion)

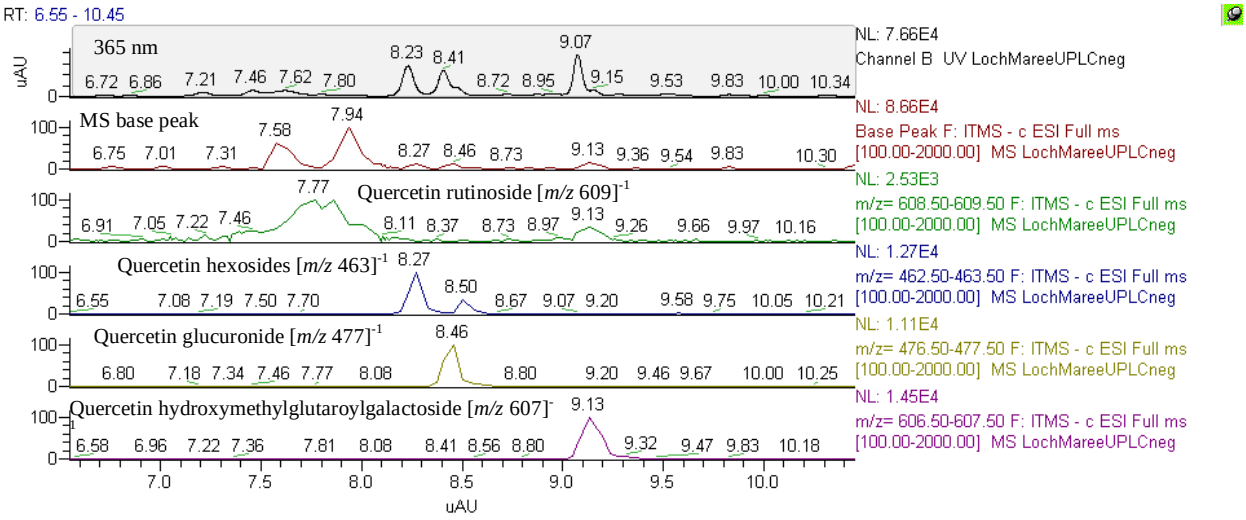




Mass chromatograms of blackberry ellagitannins (negative ion mode)



Mass chromatograms of blackberry flavonols (negative ion mode)


RT: 6.55 - 10.45
365 nm
uAU
6.72 6.86 7.21 7.46 7.62 7.80 8.23 8.41 8.72 8.95 9.07 9.15 9.53 9.83 10.00 10.34
NL: 7.66E4
Channel B UV LochMareeUPLCneg
MS base peak
6.75 7.01 7.31 7.58 7.94 8.27 8.46 8.73 9.13 9.36 9.54 9.83 10.30
NL: 8.66E4
Base Peak F: ITMS - c ESI Full ms
[100.00-2000.00] MS LochMareeUPLCneg
Quercetin rutinoside [m/z 609]-1
6.91 7.05 7.22 7.46 7.77 8.11 8.37 8.73 8.97 9.13 9.26 9.66 9.97 10.16
NL: 2.53E3
m/z= 608.50-609.50 F: ITMS - c ESI Full ms
[100.00-2000.00] MS LochMareeUPLCneg
Quercetin hexosides [m/z 463]-1
6.55 7.08 7.19 7.50 7.70 8.27 8.50 8.67 9.07 9.20 9.58 9.75 10.05 10.21
NL: 1.27E4
m/z= 462.50-463.50 F: ITMS - c ESI Full ms
[100.00-2000.00] MS LochMareeUPLCneg
Quercetin glucuronide [m/z 477]-1
6.80 7.18 7.34 7.46 7.77 8.08 8.46 8.80 9.20 9.46 9.67 10.00 10.25
NL: 1.11E4
m/z= 476.50-477.50 F: ITMS - c ESI Full ms
[100.00-2000.00] MS LochMareeUPLCneg
Quercetin hydroxymethylglutaroylgalactoside [m/z 607]-
6.58 6.96 7.22 7.36 7.81 8.08 8.41 8.56 8.80 9.13 9.32 9.47 9.83 10.18
NL: 1.45E4
m/z= 606.50-607.50 F: ITMS - c ESI Full ms
[100.00-2000.00] MS LochMareeUPLCneg
7.0 7.5 8.0 8.5 9.0 9.5 10.0
uAU

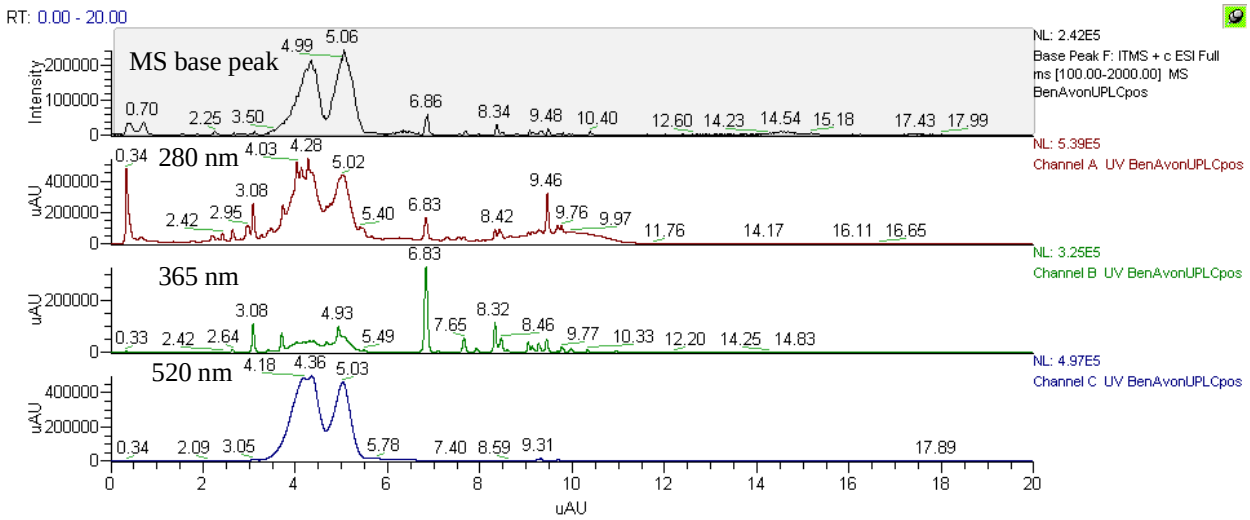
2.3.1.3 Black currant polyphenols

Black currant contains anthocyanins (analysed in positive ion mode) as major components together with significant levels of flavonols (negative and sometimes positive ion modes). The following table gives a list of polyphenols found in black currant and expected masses.

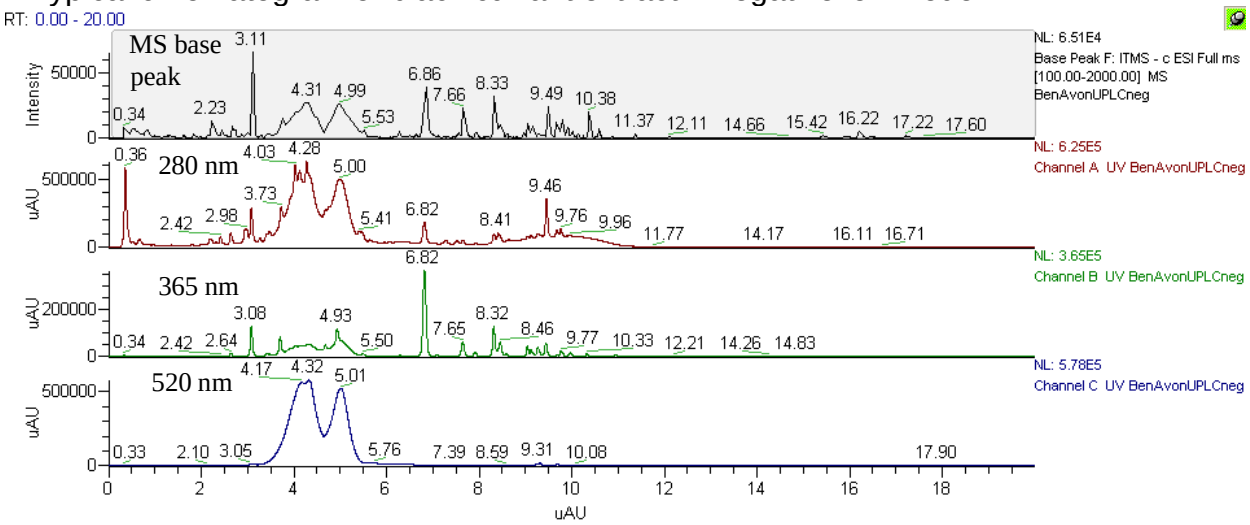
Compound	MS	MS2
Anthocyanins	**$[M+H]^+$**	
Delphinidin 3-galactoside	465	303
Delphinidin 3-glucoside	465	303
Delphinidin 3-rutinoside	611	465, 303
Cyanidin 3-glucoside	449	287
Cyanidin 3-rutinoside	595	449, 287
Petunidin 3-rutinoside	625	379, 317
Peonidin 3-galactoside	463	301
Malvidin 3-galactoside	493	331
Peonidin 3-glucoside	463	301
Peonidin 3-rutinoside	609	463, 301
Delphinidin 3-(6''-coumaroyl)glucoside		
Flavonols	**$[M-H]^-$**	
Myricetin 3-rutinoside	625	317
Myricetin 3-glucuronide	493	317
Myricetin 3-glucoside	479	317
Myricetin 3-(6''-malonyl)glucoside	565	317
Aureusidin glucoside	447	285
Quercetin 3-rutinoside	609	301
Quercetin 3-glucoside	463	301
Quercetin 3-(6''-malonyl)glucoside	549	301
Kaempferol 3-rutinoside	593	285
Kaempferol 3-galactoside	447	285
Kaempferol 3-glucoside	447	285
Isohamnetin 3-rutinoside	623	315

The following chromatograms show typical black currant (Ben Avon) profiles and identification of some major components.

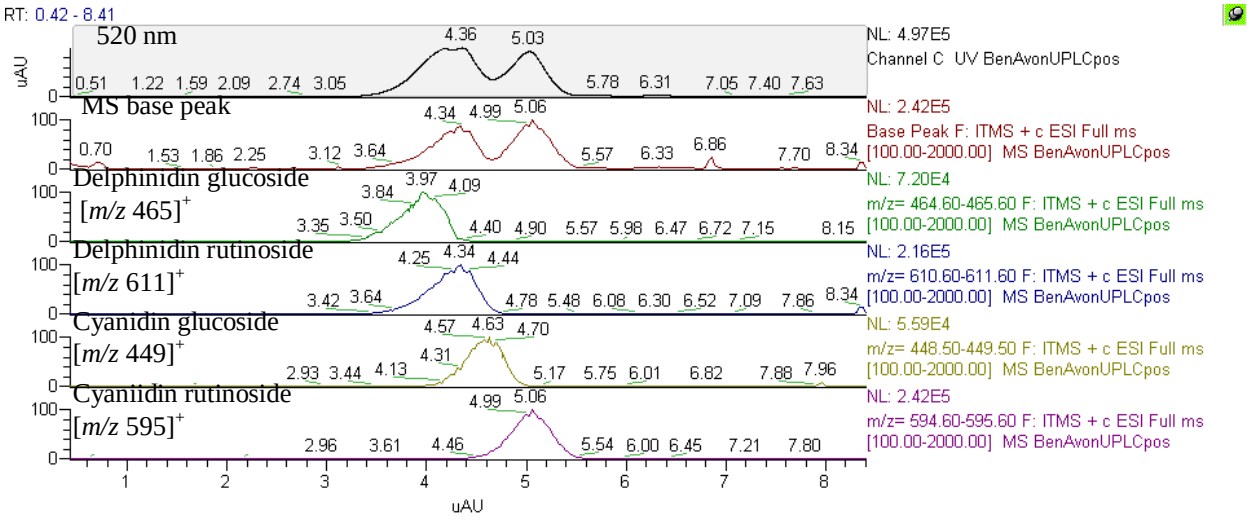
Typical chromatogram of black currant extract – positive ion mode



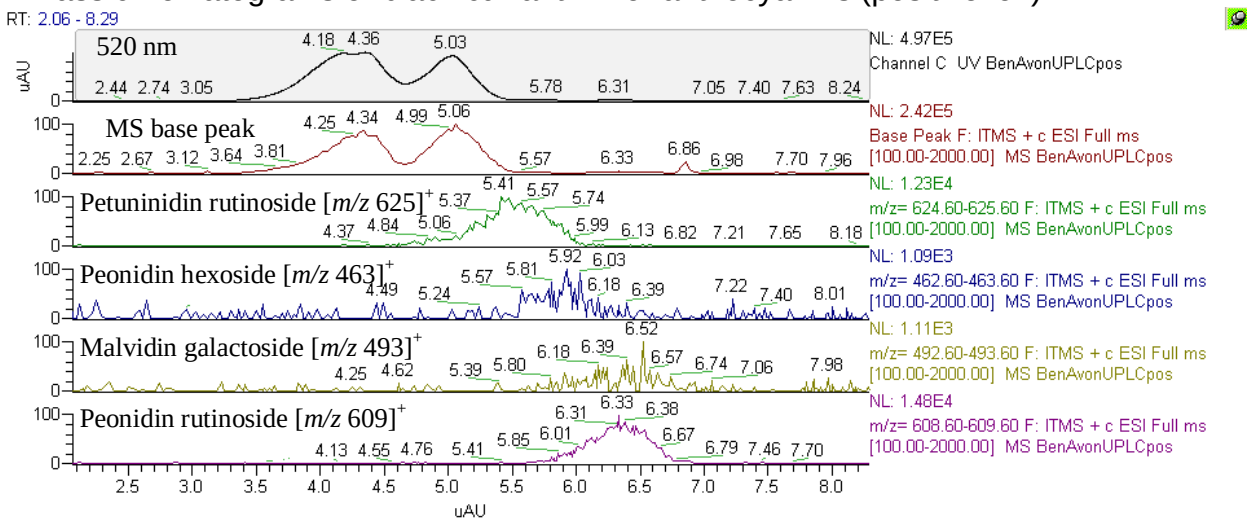
Typical chromatogram of black currant extract – negative ion mode



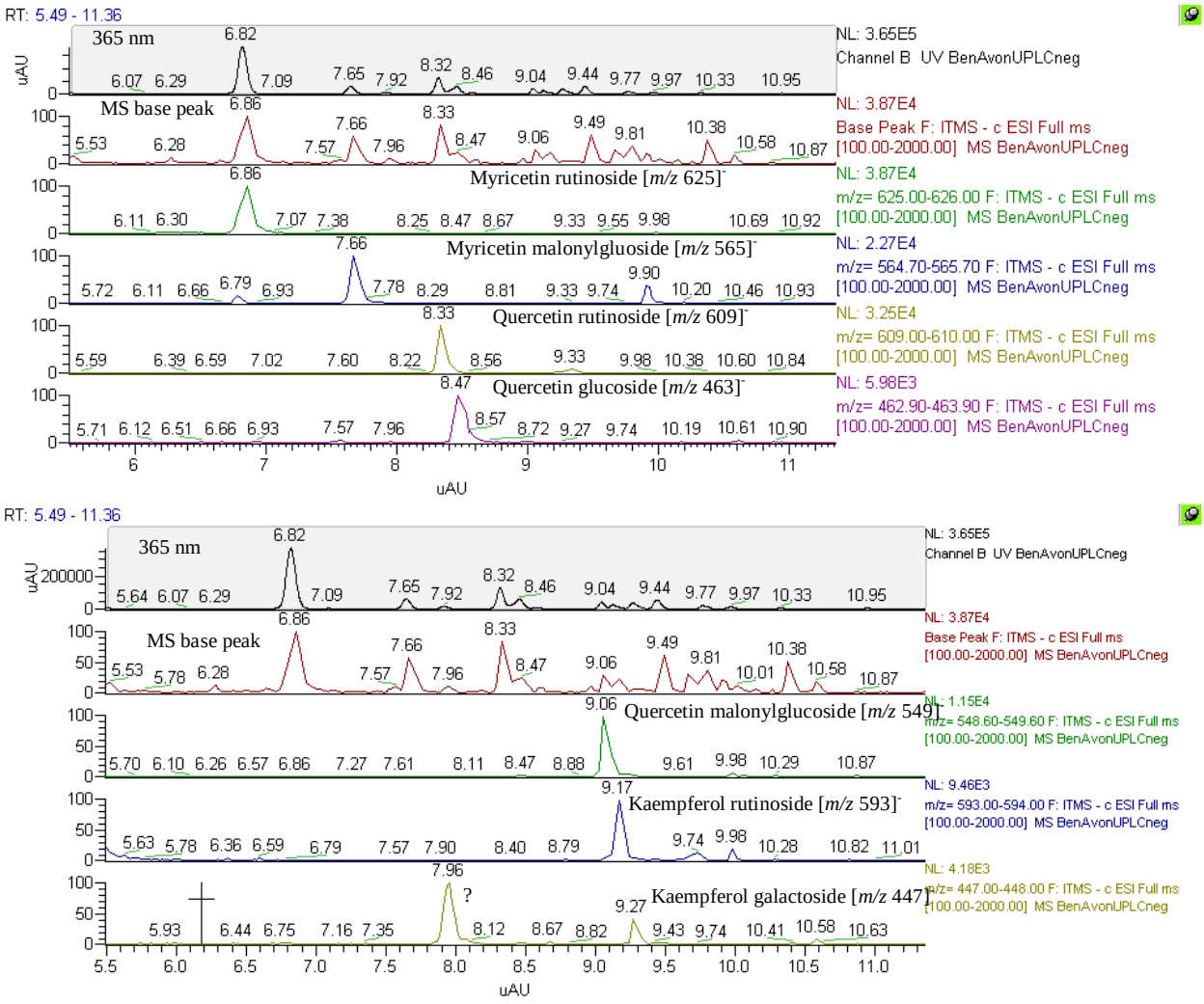
Mass chromatograms of black currant major anthocyanins (positive ion)



Mass chromatograms of black currant minor anthocyanins (positive ion)



Mass chromatograms of black currant flavonols (negative ion)



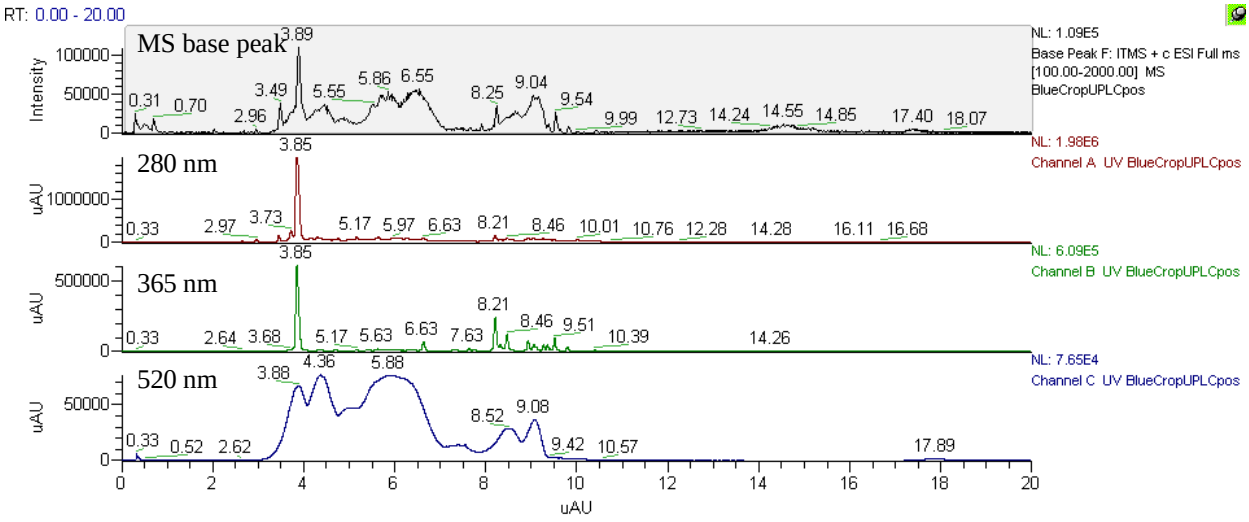
2.3.1.4 Blueberry polyphenols

Blueberry contains a complex mixture of anthocyanins (analysed in positive ion mode) and less of flavonols (negative and sometimes positive ion modes).
The following table gives a list of polyphenols found in blueberry and expected masses.

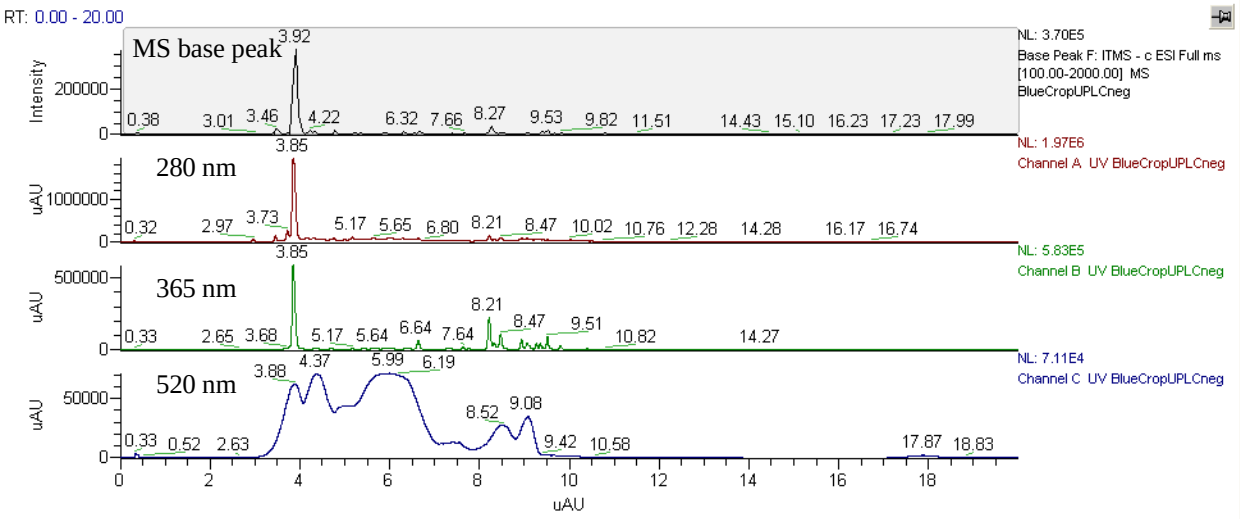
Compound	MS	MS2
Anthocyanins	**$[M+H]^+$**	
Delphinidin 3-galactoside	465	303
Delphinidin 3-glucoside	465	303
Cyanidin 3-galactoside	449	287
Delphinidin 3-arabinoside	435	303
Cyanidin 3-glucoside	449	287
Petunidin 3-galactoside	479	317
Cyanidin 3-arabinoside	419	287
Petunidin 3-glucoside	479	317
Peonidin 3-galactoside	463	301
Petunidin 3-arabinoside	449	317
Peonidin 3-glucoside	463	301
Malvidin 3-galactoside	493	331
Malvidin 3-glucoside	493	331
Peonidin 3-arabinoside	433	301
Malvidin 3-arabinoside	463	331
Cyanidin 3-(malonyl)glucoside	535	287
Cyanidin 3-(6'-acetyl)galactoside	491	287
Malvidin acetylhexoside	535	331
Petunidin pentoside	449	317
Delphinidin 3-(malonyl)glucoside	551	303
Malvidin 3-(malonyl)glucoside	579	331
Delphinidin 3-(6'-acetyl)glucoside	507	303
Peonidin 3-(6'-acetyl)galactoside	505	301
Cyanidin 3-(6'-acetyl)glucoside	491	287
Malvidin 3-(malonyl)galactoside	535	331
Petunidin 3-(6'-acetyl)glucoside	521	317
Peonidin 3-(6'-acetyl)glucoside	505	301
Malvidin 3-(6'-acetyl)glucoside	535	331
Flavonols	**$[M-H]^-$**	
Myricetin 3-galactoside	479	317
Quercetin diglucoside	625	301
Quercetin 3-rutinoside	609	301
Quercetin 3-galactoside	463	301
Quercetin 3-methoxyhexoside		
Quercetin 3-glucoside	463	301
Quercetin 3-arabinoside	433	301
Quercetin glucuronide	477	301
Quercetin 3-glucosylpentoside	595	433, 301
Quercetin 3-caffeoylgalactoside	623	463, 301
Quercetin 3-caffeoylglucoside	623	463, 301
Quercetin 3-oxalylpentoside	505	433, 301
Quercetin 3-rhamnoside	447	301
Quercetin 3-dimethoxyrhamnoside	507	477, 447, 301
Quercetin 3-(6'-acetyl)galactoside	505	463, 301
Quercetin 3-(6'-acetyl)glucoside	505	463, 301
Quercetin	301	

The following chromatograms show typical blueberry (Blue Crop) profiles and identification of some major components.

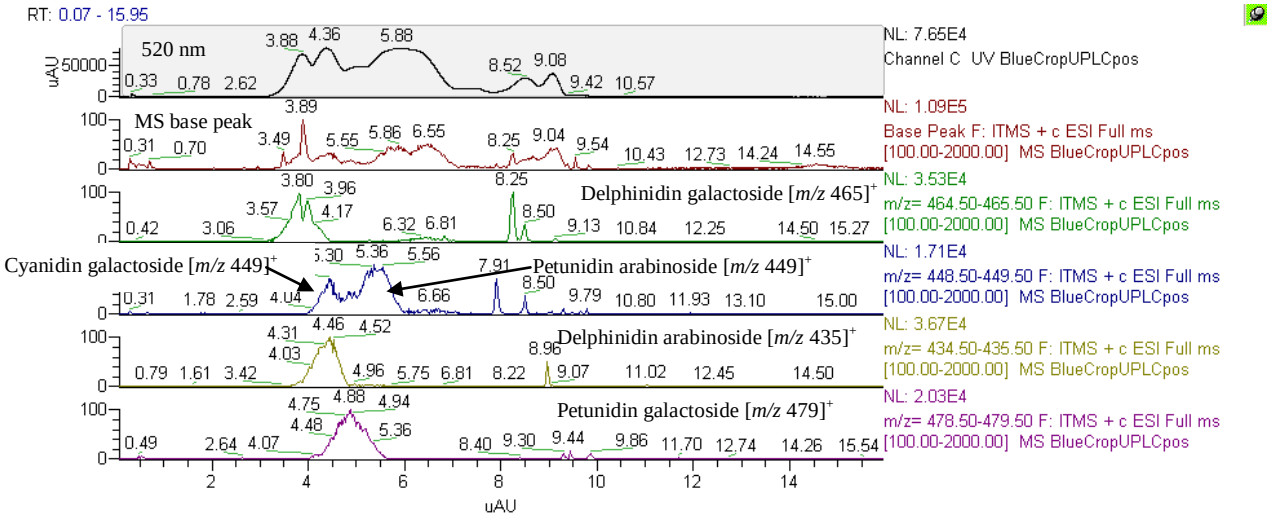
Typical chromatogram of blueberry extract – positive ion mode

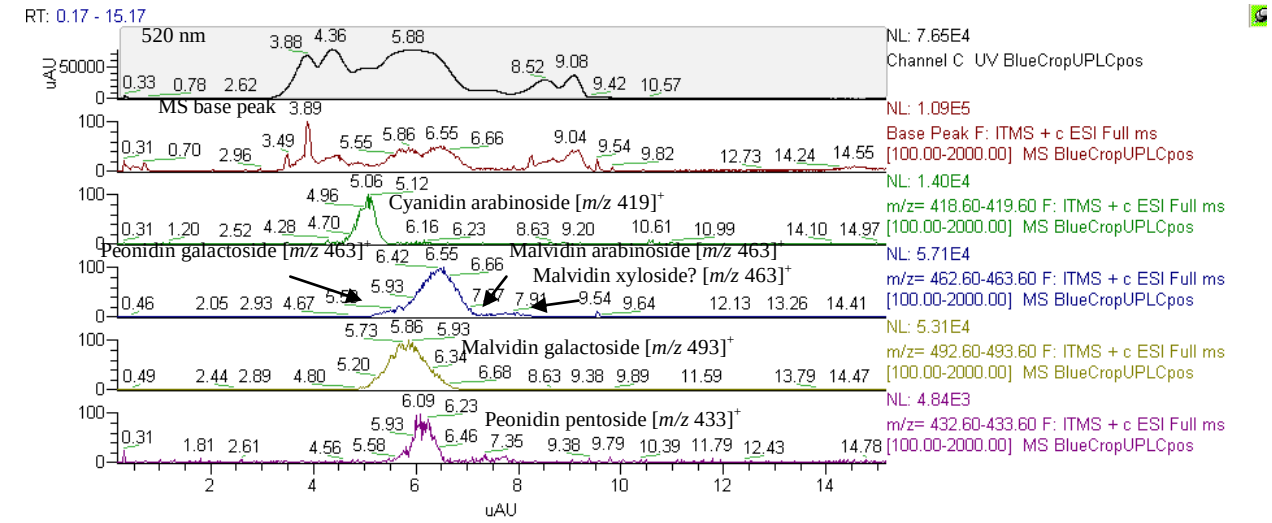


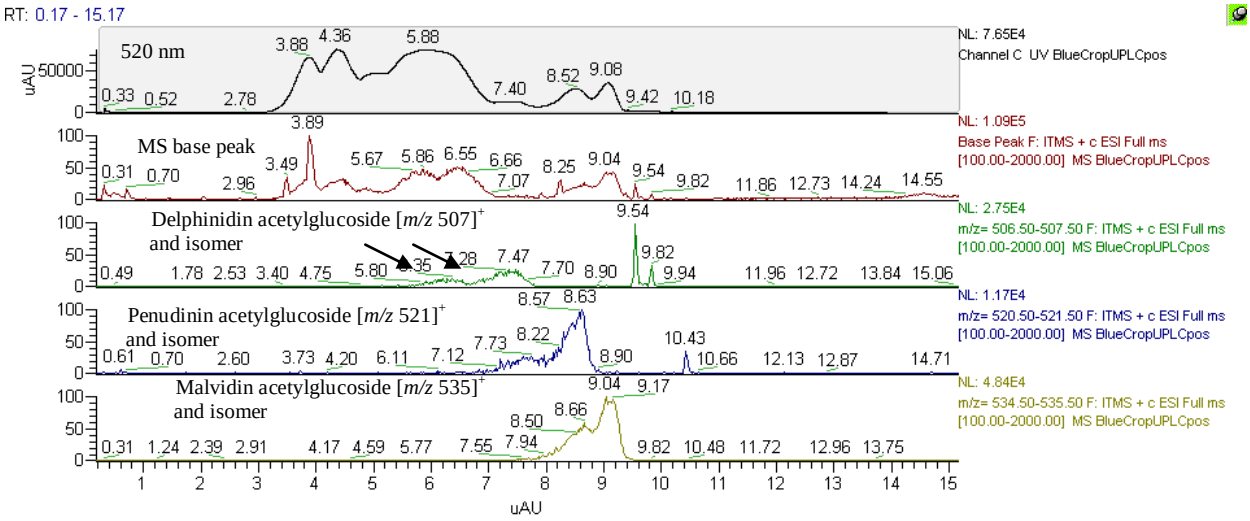
Typical chromatogram of blueberry extract – negative ion mode



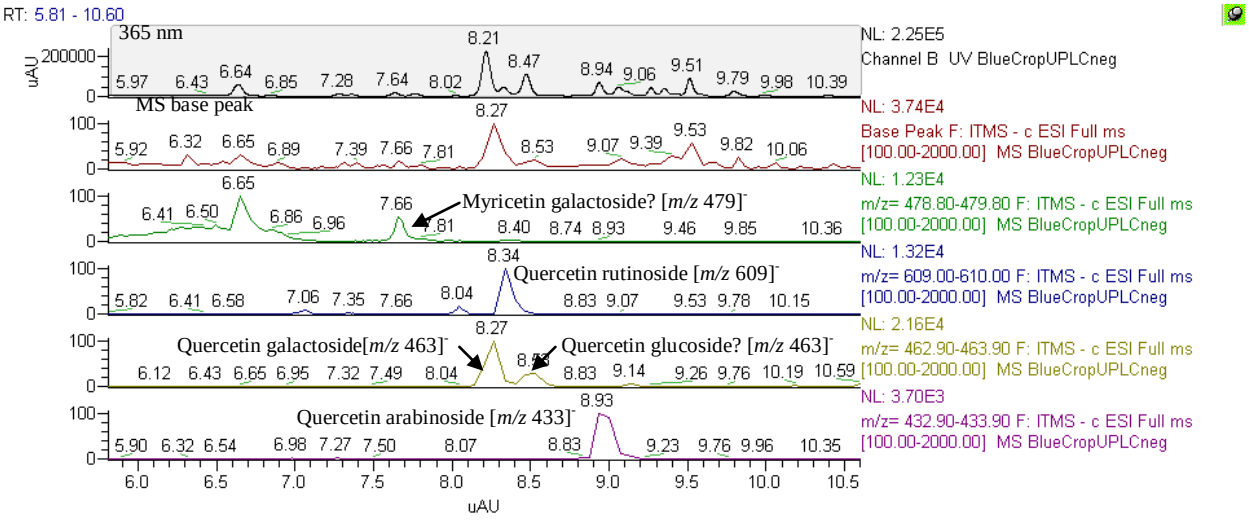
Mass chromatograms of blueberry anthocyanins (positive ion)







Mass chromatograms of blueberry flavonols (negative ion)


RT: 5.81 - 10.60
365 nm
NL: 2.25E5
Channel B UV BlueCropUPLCneg
MS base peak
NL: 3.74E4
Base Peak F: ITMS - c ESI Full ms
[100.00-2000.00] MS BlueCropUPLCneg
NL: 1.23E4
m/z= 478.80-479.80 F: ITMS - c ESI Full ms
[100.00-2000.00] MS BlueCropUPLCneg
Myricetin galactoside? [m/z 479]-
NL: 1.32E4
m/z= 609.00-610.00 F: ITMS - c ESI Full ms
[100.00-2000.00] MS BlueCropUPLCneg
Quercetin rutinoside [m/z 609]-
NL: 2.16E4
m/z= 462.90-463.90 F: ITMS - c ESI Full ms
[100.00-2000.00] MS BlueCropUPLCneg
Quercetin galactoside[m/z 463]-
Quercetin glucoside? [m/z 463]-
NL: 3.70E3
m/z= 432.90-433.90 F: ITMS - c ESI Full ms
[100.00-2000.00] MS BlueCropUPLCneg
Quercetin arabinoside [m/z 433]-
uAU
uAU

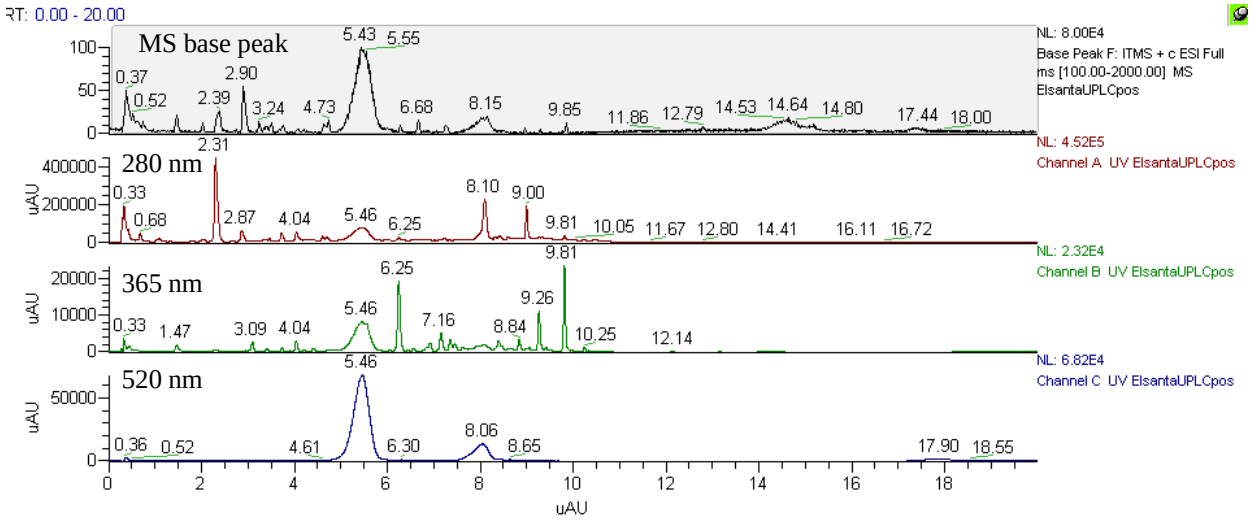
2.3.1.5 Strawberry polyphenols

Strawberry contains large amounts of proanthocyanidins and less anthocyanins (analysed in positive ion mode) and ellagitannins (negative ion mode). Flavonols (negative and sometimes positive ion modes) are minor components.
The following table gives a list of polyphenols found in strawberry and expected masses.

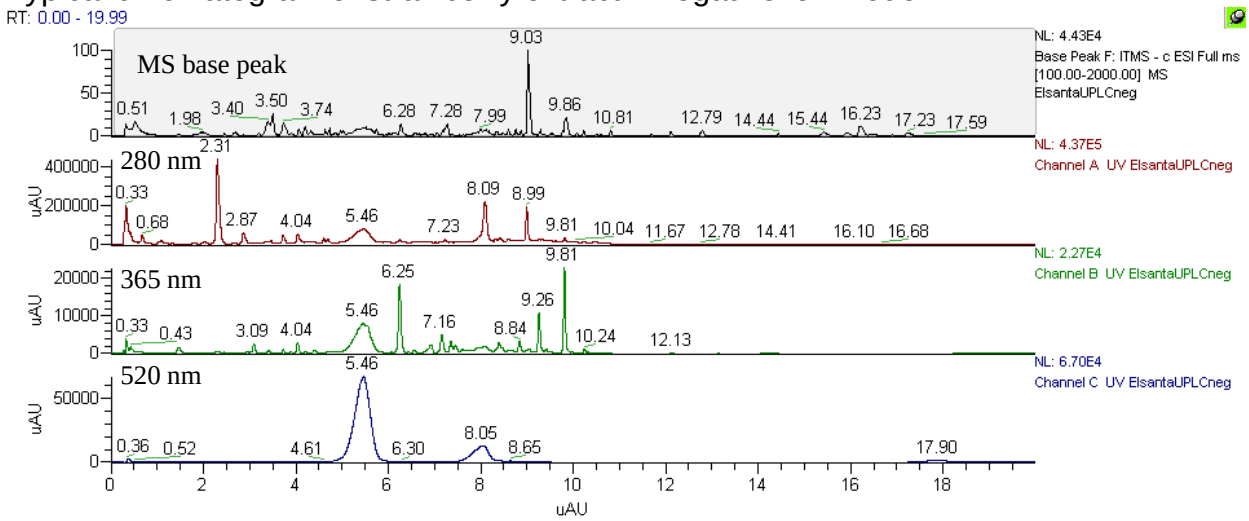
Compound	MS	MS2
Anthocyanins	**$[M+H]^+$**	
Cyanidin 3-sophoroside	611	287
Cyanidin 3-glucoside	449	287
Cyanidin 3-rutinoside	595	449, 287
Pelargonidin 3-glucoside	433	271
Petunidin 3-glucoside	479	317
Pelargonidin 3-rutinoside	579	433, 271
Pelargonidin 3-(malonyl)glucoside	519	433, 271
Pelargonidin 3-(6'-acetyl)glucoside	475	271
Flavonol	**$[M-H]^-$**	
Quercetin 3-glucuronide	477	301
Kaempferol 3-coumaroylglucoside	593	447, 285
Kaempferol 3-glucoside	447	285
Kaempferol 3-acetylglucoside	489	285
Ellagitannins/ellagic acid derivatives	**$[M-H]^-$**	
Bis-HDDP-glucoside	783	301
Galloyl bis-HHDP-glucoside 1	935	633, 301
Galloyl bis-HHDP-glucoside 1	935	633, 301
Sanguiin-H6	$[1869]^{-1}$ $[935]^{-1}$	1697, 1567, 1407,897, 783, 633, 301
Lambertianin C	$[1401]^{-2}$	2019, 1869, 1567, 1402, 935, 897, 633, 301
Ellagic rhamnoside 1	447	301
Ellagic rhamnoside 2	447	301
Flavan-3-ols/proanthocyanidins	**$[M-H]^-$**	
Dimer (Cat-Cat)	577	
Dimer (Cat-Afz)	561	
Trimer (Cat-Cat-Cat)	865	
Trimer (Cat-Cat-Afz)	849	
Trimer Cat-Afz-Afz)	833	
Tetramer (Cat-Cat-Cat-Cat)	1153	
Tetramer (Cat-Cat-Cat-Afz)	1137	

The following chromatograms show typical strawberry (Elsanta) profiles and identification of some major components.

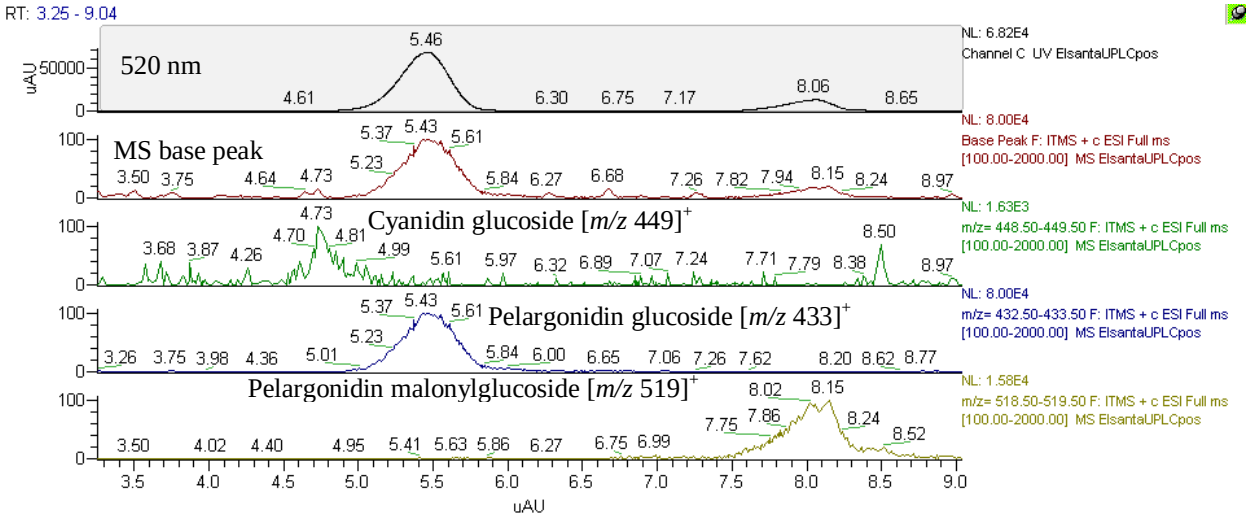
Typical chromatogram of strawberry extract – positive ion mode



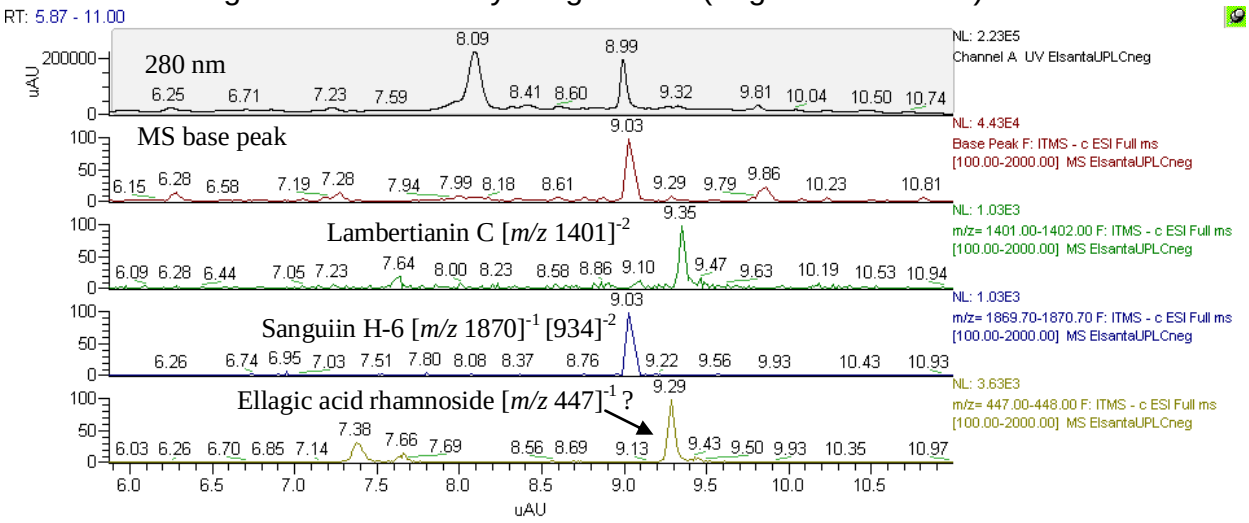
Typical chromatogram of strawberry extract – negative ion mode



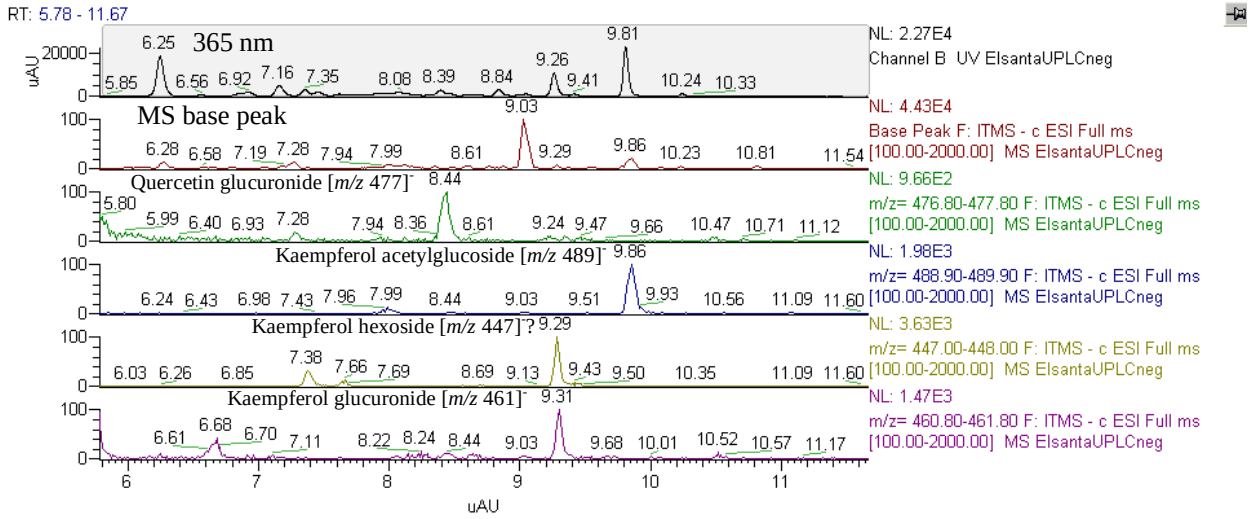
Mass chromatograms of strawberry anthocyanins (positive ion)



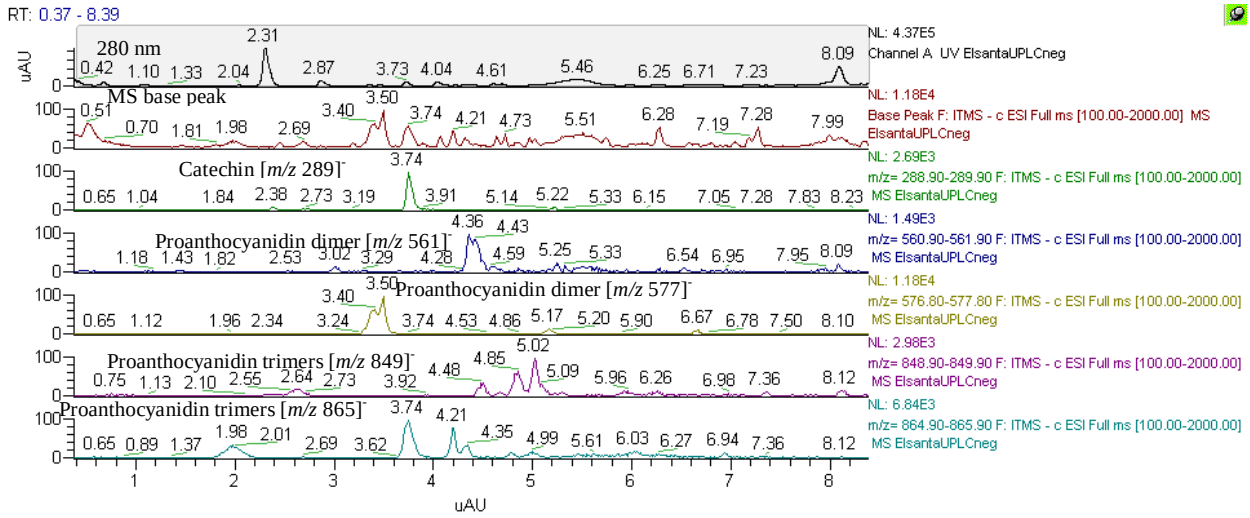
Mass chromatograms of strawberry ellagitannins (negative ion mode)

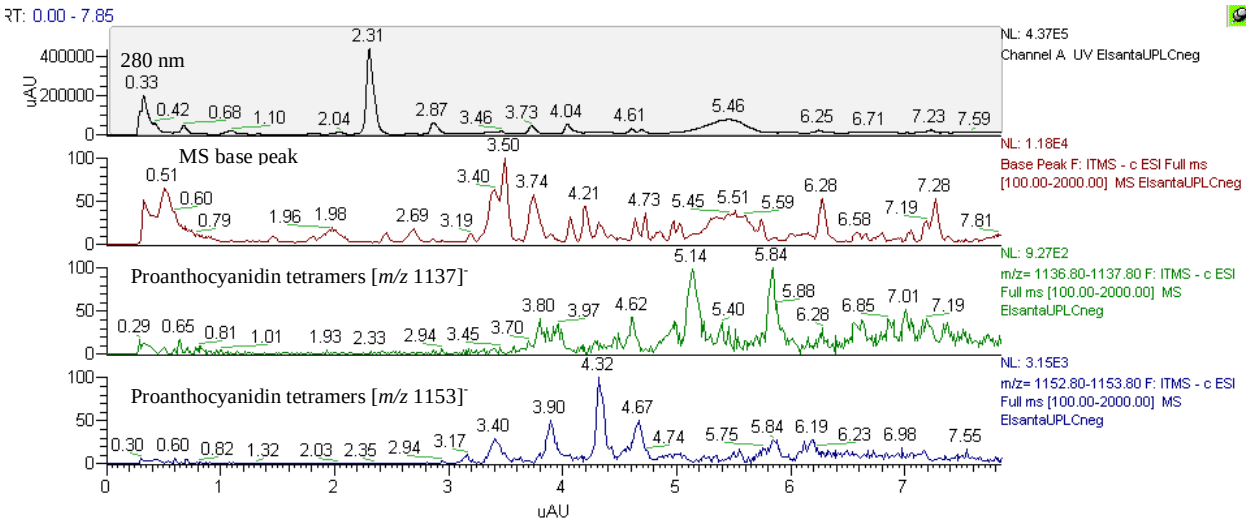


Mass chromatograms of strawberry flavonols (negative ion mode)



Mass chromatograms of strawberry flavan-3-ols (negative ion mode)


RT: 0.37 - 8.39
280 nm
MS base peak
Catechin [m/z 289]-
Proanthocyanidin dimer [m/z 561]-
Proanthocyanidin dimer [m/z 577]-
Proanthocyanidin trimers [m/z 849]-
Proanthocyanidin trimers [m/z 865]-
uAU
NL: 4.37E5
Channel A UV ElsantaUPLCneg
NL: 1.18E4
Base Peak F: ITMS - c ESI Full ms [100.00-2000.00] MS ElsantaUPLCneg
NL: 2.69E3
m/z= 288.90-289.90 F: ITMS - c ESI Full ms [100.00-2000.00] MS ElsantaUPLCneg
NL: 1.49E3
m/z= 560.90-561.90 F: ITMS - c ESI Full ms [100.00-2000.00] MS ElsantaUPLCneg
NL: 1.18E4
m/z= 576.80-577.80 F: ITMS - c ESI Full ms [100.00-2000.00] MS ElsantaUPLCneg
NL: 2.98E3
m/z= 848.90-849.90 F: ITMS - c ESI Full ms [100.00-2000.00] MS ElsantaUPLCneg
NL: 6.84E3
m/z= 864.90-865.90 F: ITMS - c ESI Full ms [100.00-2000.00] MS ElsantaUPLCneg

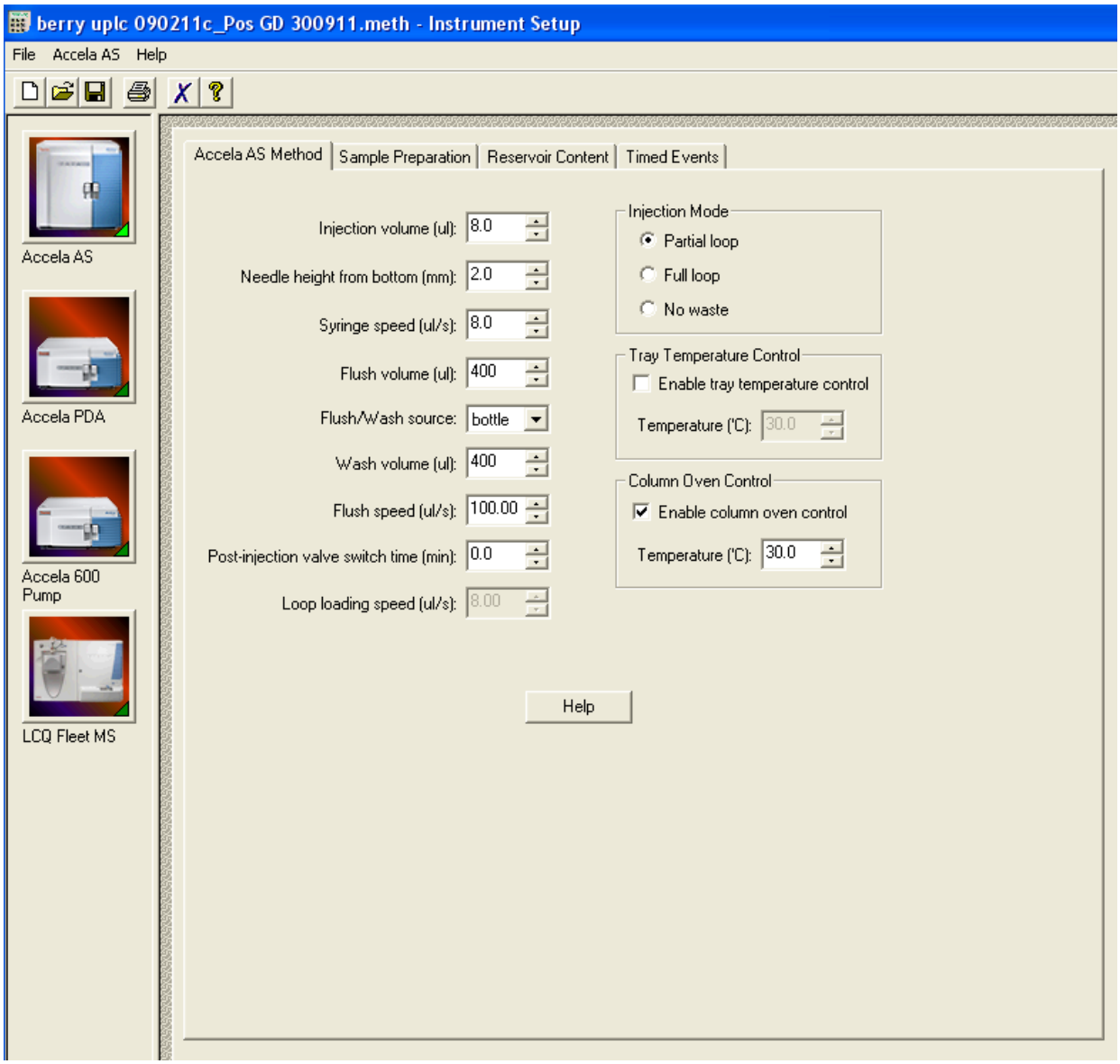

RT: 0.00 - 7.85
280 nm
MS base peak
Proanthocyanidin tetramers [m/z 1137]-
Proanthocyanidin tetramers [m/z 1153]-
uAU
NL: 4.37E5
Channel A UV ElsantaUPLCneg
NL: 1.18E4
Base Peak F: ITMS - c ESI Full ms [100.00-2000.00] MS ElsantaUPLCneg
NL: 9.27E2
m/z= 1136.80-1137.80 F: ITMS - c ESI Full ms [100.00-2000.00] MS ElsantaUPLCneg
NL: 3.15E3
m/z= 1152.80-1153.80 F: ITMS - c ESI Full ms [100.00-2000.00] MS ElsantaUPLCneg

2.3.2 Quantification

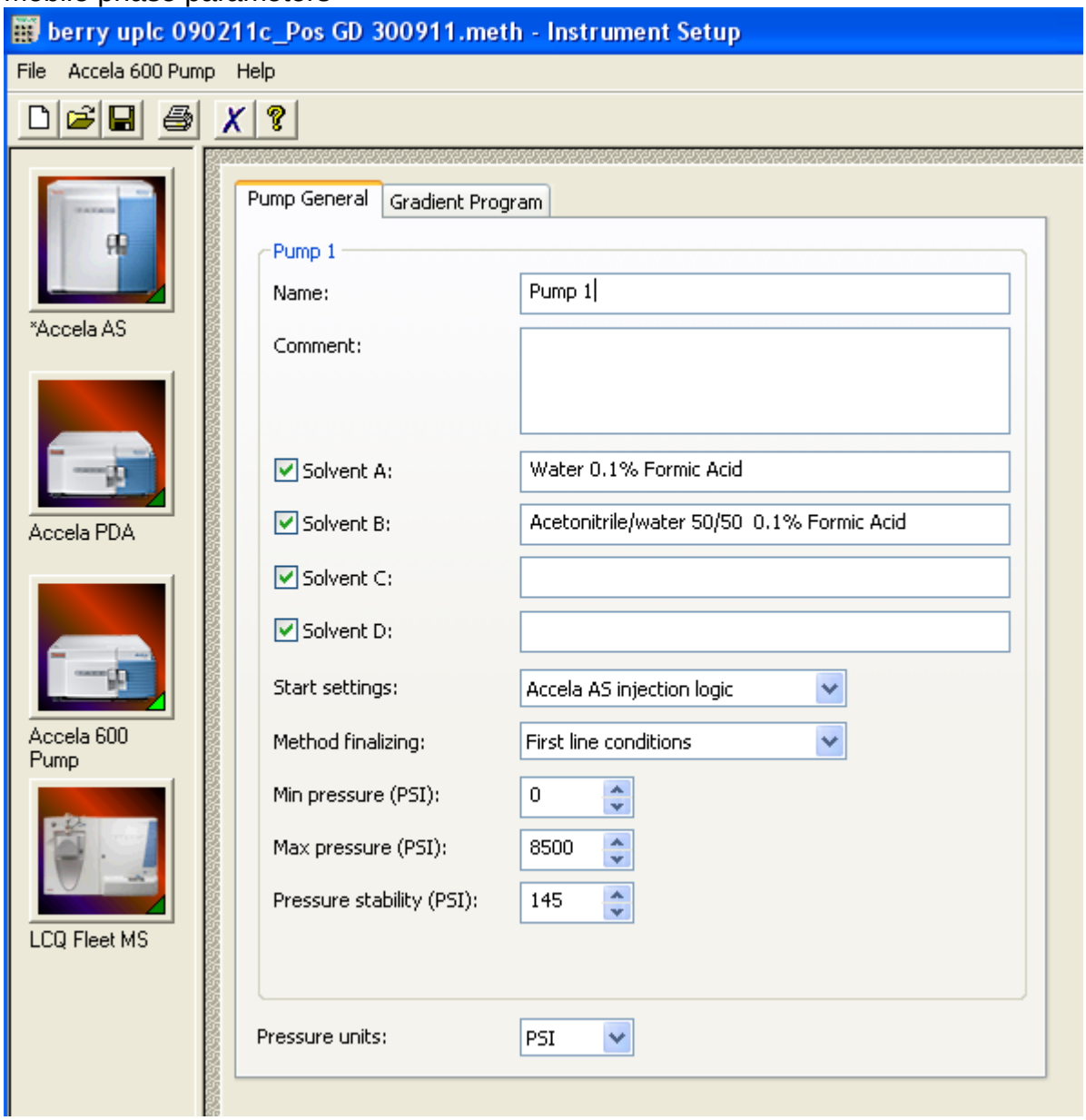
For quantification of compounds, morin is added to the juice sample at a concentration of 0.1 mg mL^{-1} (add 100 μl of 1 mg mL^{-1} morin to 900 μl juice). Suitable external standards similar to the compounds of interest are prepared to cover the concentrations in the juice. Morin is added to each standard at the same concentration (0.1 mg mL^{-1}) as in the juice and the standards are analysed together with samples. Samples and standards are processed so that the ratio of the area of a specific ion of the compound of interest to that for morin internal standard (*m*/*z* 301 and 303 for negative and positive mode, respectively) is calculated. In Excel, calibration curves of response ratios against concentration are plotted for the standards and best-fit equations are used to calculate the concentrations in the juices.

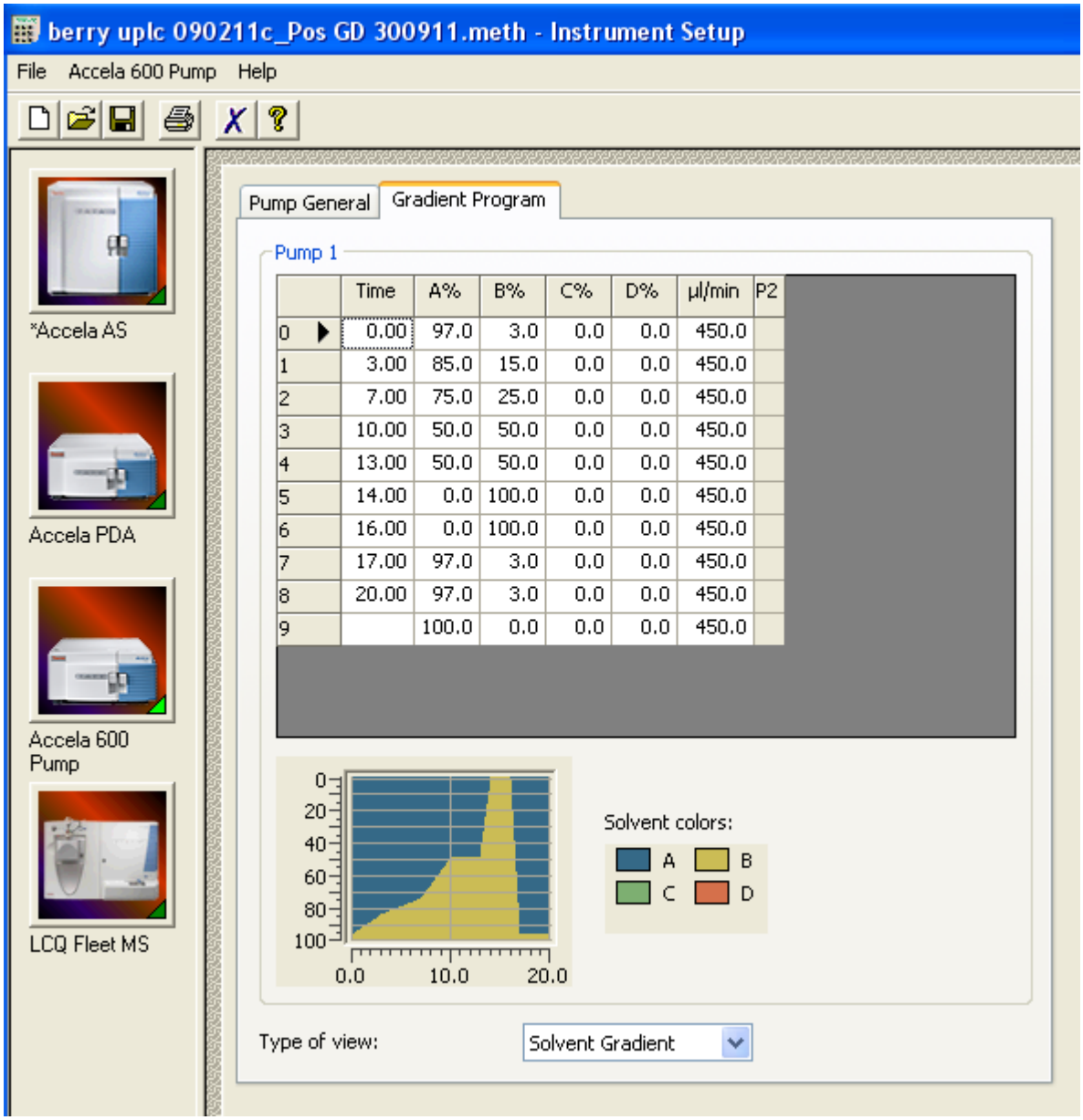
Appendix. UPLC-PDA-MS instrument parameters

Autosampler parameters

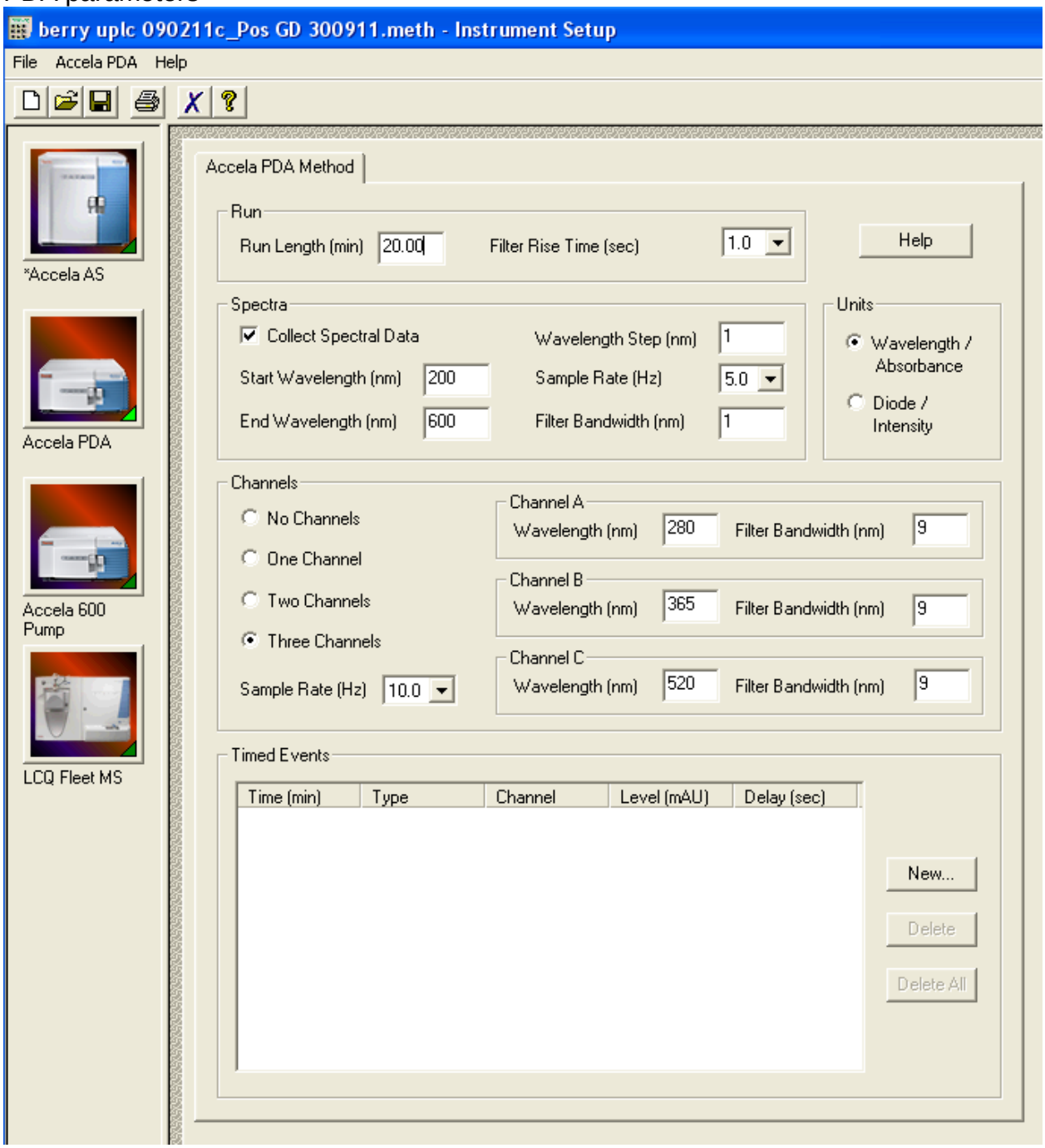


Mobile phase parameters

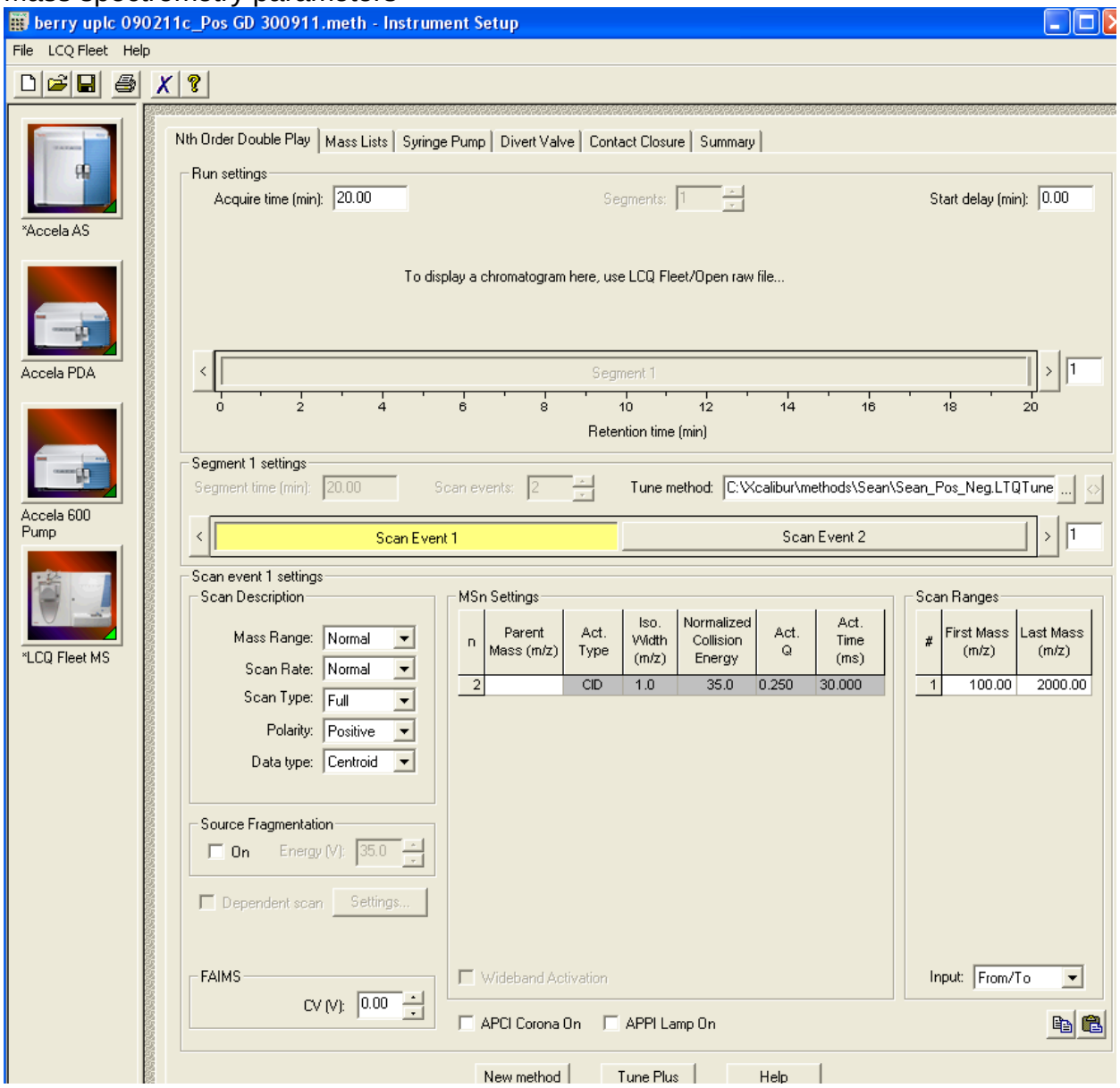



berry uplc 090211c_Pos GD 300911.meth - Instrument Setup
File
Accela 600 Pump
Help
*Accela AS
Accela PDA
Accela 600 Pump
LCQ Fleet MS
Pump General
Gradient Program
Pump 1
Time A% B% C% D% µl/min P2
0 0.00 97.0 3.0 0.0 0.0 450.0
1 3.00 85.0 15.0 0.0 0.0 450.0
2 7.00 75.0 25.0 0.0 0.0 450.0
3 10.00 50.0 50.0 0.0 0.0 450.0
4 13.00 50.0 50.0 0.0 0.0 450.0
5 14.00 0.0 100.0 0.0 0.0 450.0
6 16.00 0.0 100.0 0.0 0.0 450.0
7 17.00 97.0 3.0 0.0 0.0 450.0
8 20.00 97.0 3.0 0.0 0.0 450.0
9 100.0 0.0 0.0 0.0 450.0
0
20
40
60
80
100
0.0
10.0
20.0
Solvent colors:
A
B
C
D
Type of view:
Solvent Gradient

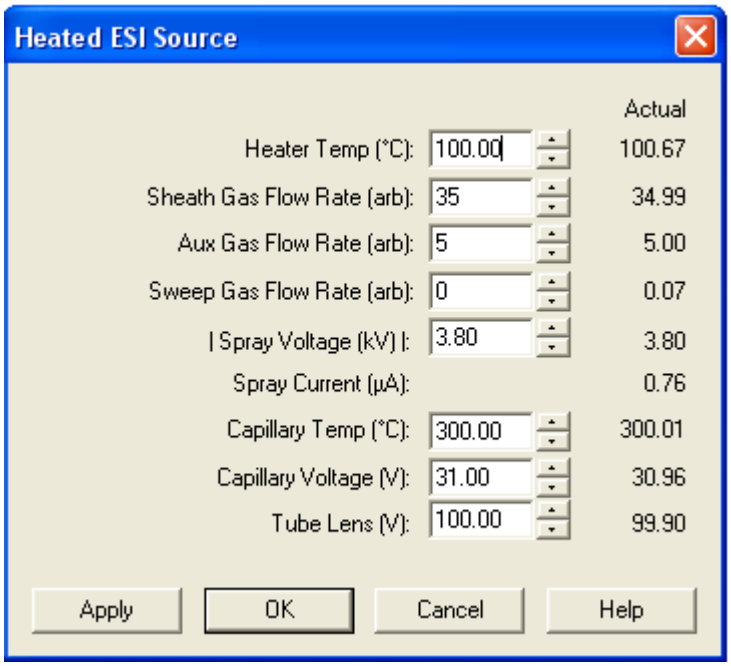
PDA parameters



Mass spectrometry parameters



Souce parameters (tune file)


Heated ESI Source
Actual
Heater Temp (°C):
100.00
100.67
Sheath Gas Flow Rate (arb):
35
34.99
Aux Gas Flow Rate (arb):
5
5.00
Sweep Gas Flow Rate (arb):
0
0.07
| Spray Voltage (kV) |:
3.80
3.80
Spray Current (µA):
0.76
Capillary Temp (°C):
300.00
300.01
Capillary Voltage (V):
31.00
30.96
Tube Lens (V):
100.00
99.90
Apply
OK
Cancel
Help

METHODOLOGIES P12

ANALYSIS OF SENSORIAL PARAMETERS

A) HUMAN SENSORY

RASPBERRY - SOP for human-sensory test

1) Principle: Combined sensory test including profile analysis and modified preference test. The method is designed for rapid testing of cultivars, breeding clones and wild types.

2) Panel: Expert panel consisting of minimal 10 testers.

3) Sample preparation: Human-sensory parameters are tested immediately after harvest. Fruits are washed with tap water, dried with paper towels and sepals are removed. A minimum of 1 kg fruits are mixed to get a representative sample and and placed inside a glass bowl with cover plate. The bowls are prepared not less than 15 minutes before testing to allow saturation of head-space in the bin.

4) Sensory test: The panelists quantified the features by tagging the intensity on the 9-step graduated scale. Tap water is used for neutralisation.

5) Sensory form number F.C.0029: Established using the software FIZZ forms from Biosystemes (France) version 2.45 A.

6) Reference: Hoberg,E.; Ulrich,D.; Schulz,H.; Tuvia-Alkali,S.; Fallik,E. (2003) Sensory and quality analysis of different melon cultivars after prolonged storage. Nahrung-Food, 47 (5) 320-324

STRAWBERRY - SOP for human-sensory test

1) Principle: Combined sensory test including profile analysis and modified preference test. The method is designed for rapid testing of cultivars, breeding clones and wild types.

2) Panel: Expert panel consisting of minimal 10 testers.

3) Sample preparation: Human-sensory parameters are tested immediately after harvest. Fruits are washed with tap water, dried with paper towels, sepals are removed and fruits are cutted at the polar axis in four sections. The sections of minimal 1 kg fruits are mixed to get a representative sample and placed inside a glass bowl with cover plate. The bowls are prepared not less than 15 minutes before testing to allow saturation of head-space in the bin.

4) Sensory test: The panelists quantified the features by tagging the intensity on the 9-step graduated scale. Tap water is used for neutralisation.

5) Sensory form F.C.0030: The form was established using the software FIZZ forms from Biosystemes (France), version 2.45 A.

6) Reference: Hoberg,E.; Ulrich,D.; Schulz,H.; Tuvia-Alkali,S.; Fallik,E. (2003) Sensory and quality analysis of different melon cultivars after prolonged storage. Nahrung-Food, 47 (5) 320-324

Sensory form for raspberries:

F.C. 0029 O.N. 000:1 PAGE 00:1/0:10

Raspberry - Sensory Evaluation

Judge:
Date:

Sample no:

	low								high
raspberry odour	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
untypical odour	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
sweet	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
sour	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
harmonical	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
raspberry aroma	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
green / grassy	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
fruity (others)	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
untypical	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
juicy	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
firmness	☐	☐	☐	☐	☐	☐	☐	☐	☐
	1				5				9
	low								high
preference	☐	☐	☐	☐	☐	☐	☐	☐	☐
	1	2	3	4	5	6	7	8	9

Comments:

Sensory form for strawberries:

F.C. ☐☐3☐ O.N. ☐☐☐:| PAGE ☐☐:|/☐:|☐

Strawberry - Sensory Evaluation

Judge:
Date:

Sample no:

	low								high
strawberry odour	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
untypical odour	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
sweet	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
sour	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
harmonical	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
strawberry aroma	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
green / grassy	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
fruity (others)	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
untypical	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
vesca-like	☐	☐	☐	☐	☐	☐	☐	☐	☐
	low 1				5				9 high
firmness	☐	☐	☐	☐	☐	☐	☐	☐	☐
	1				5				9
	low								high
preference	☐	☐	☐	☐	☐	☐	☐	☐	☐
	1	2	3	4	5	6	7	8	9

Comments:

B) Semi-quantitative analysis of aroma patterns by gas

Chromatography

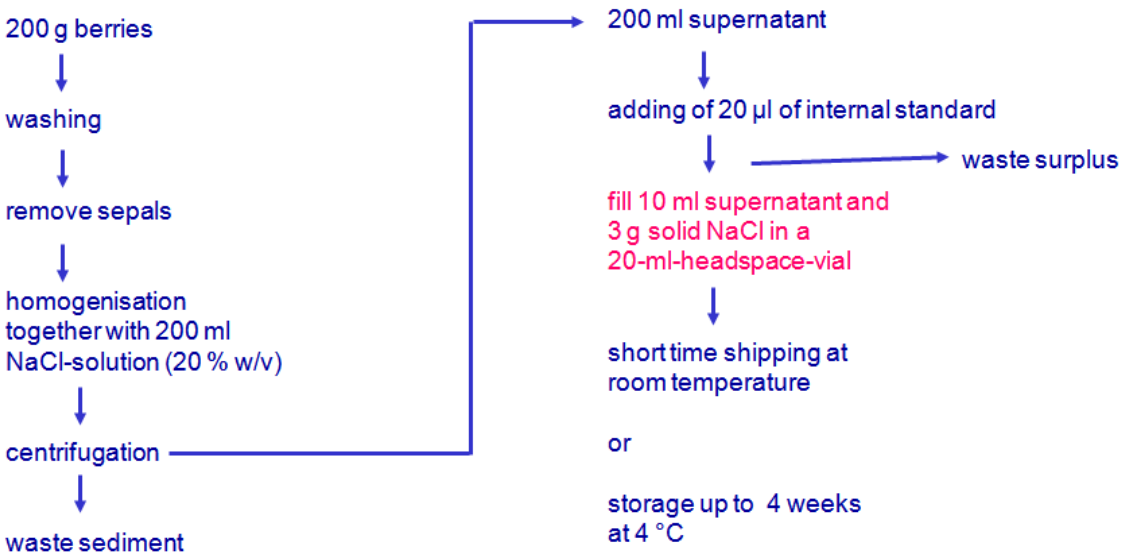
RASPBERRY AND STRAWBERRY

A minimum of 200 g washed, fresh fruits without sepals were homogenized at room temperature for 1 min in a Waring Blendor (high speed) together with a NaCl solution (20% w/v). The ratio of fruit weight to the volume of NaCl solution was 1: 1 w/v. The homogenate was centrifuged at 4 °C and 3,500 rpm for 30 min and additionally filtered using gossamer. Headspace vials containing 3 g of solid NaCl for saturation were filled with a 10 mL aliquot of the supernatant and sealed with a magnetic crimp cap including septum. Flow sheets for three different sample sizes are appended.

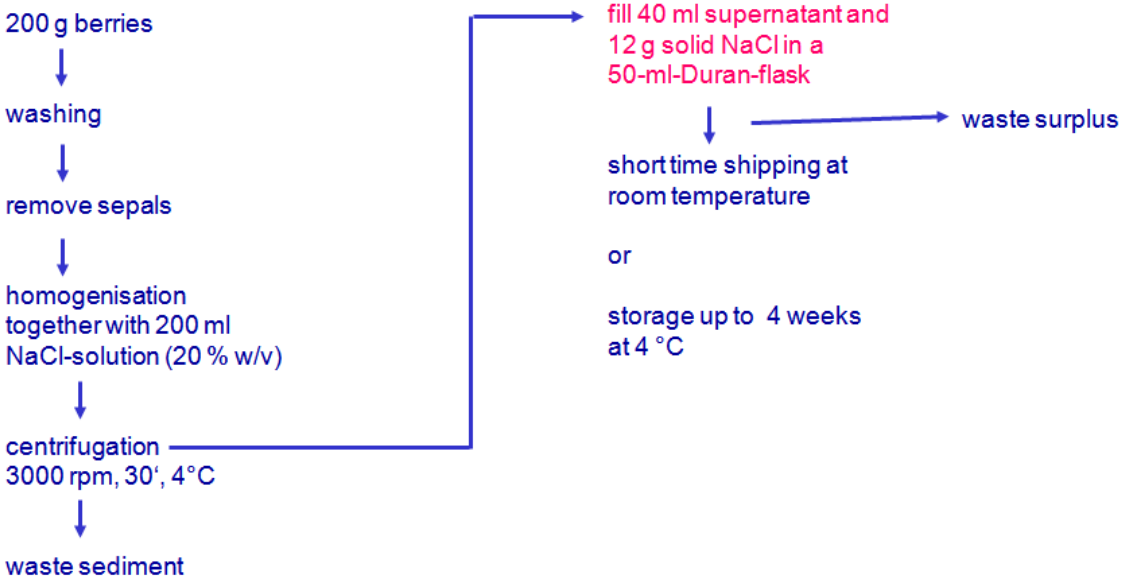
For automated headspace-SPME-GC a 100 µmpolydimethylsiloxane fiber (Supelco, Bellefonte, PA) was used. After an equilibration time of 10 min at 35 °C (300 rpm), the extraction of volatiles persisted for 15 min at 35 °C. Desorption was 2 min in splitless mode and 3 min with split at 250 °C. An Agilent Technologies 6890 GC equipped with an MPS2 autosampler from Gerstel (Muehlheim, Germany), an HP-INNOWax column (0.25 mm i.d., 30 m length, and 0.5 µmfilm thickness), and FID was used for chromatography. Carrier gas was hydrogen with a flow rate of 1.1 mL/min. The temperature program was the following: 45 °C (5 min), from 45 to 210 °C at 5 K/min, and 15 min at 200 °C. The volatiles were identified by parallel running of selected samples on a GC-MS (EI mode, m/z range from 35 to 350 amu) with library search (NIST and MassFinder) and by retention indices as well as coelution of authentic references (selected compounds).

The commercial software Chromstat version 2.6 by AnalytMuellheim (Germany) was used for data processing by pattern recognition (nontargeted or holistic analysis approach). Data input for pattern recognition are raw data from the percentage reports (retention time/peak area data pairs) performed with the software package Chemstation by Agilent. Using Chromstat, the chromatograms were divided in up to 200 time intervals, each of which represents a possible peak (substance) occurring in at least one chromatogram of the analysis set. The peak detection threshold was set to the 10-fold value of background noise. Semi-quantification is done by using absolute peak areas, norm percent calculation or a internal standard procedure.

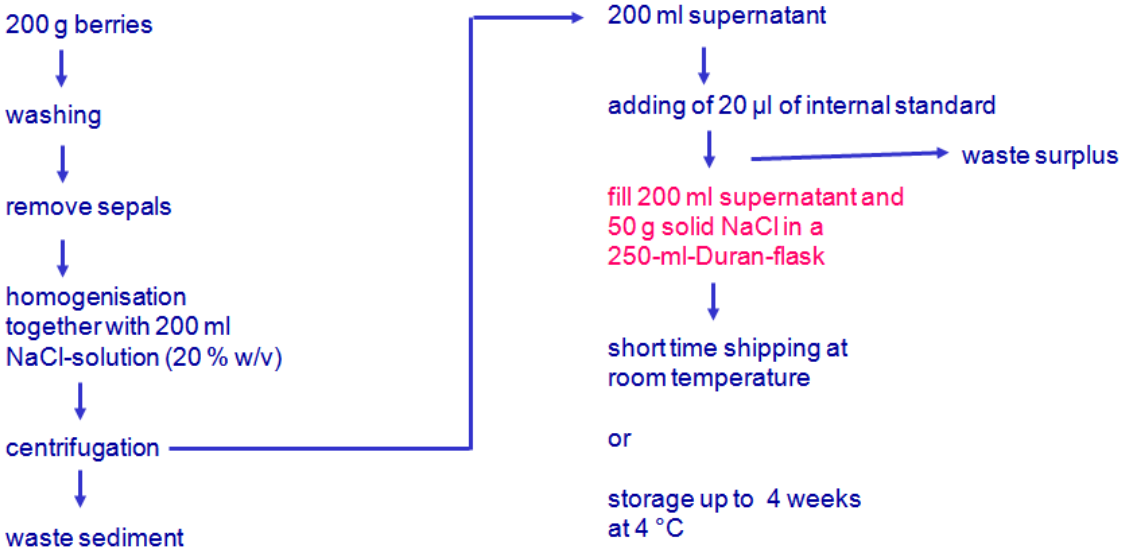
Standard sample preparation for RASPBERRY
Headspace solid phase microextraction (HS-SPME-GC) and Immersion-SBSE
(20 ml headspace vials)



Standard sample preparation for RASPBERRY (JKI OePV SOP 047)
Headspace solid phase microextraction (HS-SPME-GC) and Immersion-SBSE
(50 ml Duran flask)



Standard sample preparation for RASPBERRY (JKI OePV SOP 047)
Headspace solid phase microextraction (HS-SPME-GC) and Immersion-SBSE
(250 ml Duran flask)



References:

D. Ulrich, T. Bruchmüller, H. Krüger and F. Marthe (2011) Sensory Characteristics and Volatile Profiles of Parsley (Petroselinum crispum [Mill.] Nym.) in Correlation to Resistance Properties against
Septoria Blight (Septoria petroselini). J. Agric. Food Chem. 59, 10651-10656.

K. Olbricht , C. Grafe , K. Weiss and D. Ulrich (2008) Inheritance of aroma compounds in a model population of Fragaria x ananassa Duch. Plant Breeding 127, 87-93.