Analyse different chilli peppers biology essay

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The purpose of this project was to analyse different chilli peppers and hot sauces for their Capsaicin and Dihydrocapsaicin content but concentration was focused more on the analysis of different chillies than sauces. Samples of chillies were refluxed using Ethanol as an extraction solvent and sauces were untrasonicated using Methanol. The extracts were filtered and analysed via Reverse phase HPLC-UV Vis technique. A number of experiments were performed to optimise the method that has been previously used for analysis of chilli peppers. The first experiment conducted was to optimise the duration of reflux time required to obtain a good yield of Capsaicin. Results showed that 1hr is sufficient for the extraction of Capsaicin. A variety of chilli peppers and sauces were analysed so as to ascertain the hottest pepper. In general all samples had good detection. Different parts of chillies were also examined to establish which part contains the highest concentration of Capsaicin and Endocarp was found to be the hottest part. The reproducibility of the method was also investigated and the sample showed to have a low RSD value.

1. Introduction

“ Next to Jazz music, there’s nothing that lifts the spirit and strengthens

the soul more than a good bowl of chillies.”

Harry James (Late American musician)

Loved by millions for their hot and sizzling flavours, the chilli peppers have become very popular over the period of time and are being grown in almost in all parts of the world, with Asia being the biggest producer of chillies followed by Mexico and the U. S.

In traditional Indian medical system, chilli is used as way of stimulating the digestion and is also believed to be a natural pain killer.

The red chilli peppers are also a source of potassium, magnesium and iron and vitamin C.

1. 1 Why Are They So Hot?

The ‘ heat’ sensation in chillies is caused by a class of chemicals, called the Capsaicinoids. These compounds are found in members of the capsicum family of plants. Capsaicinoids themselves belong to a group called ‘ Vanilloids’ i. e. containing the Vanillyl group [3dchem. com]

Figure 1. 1 [en. wikipedia. org]

All Capsaicinoids have same functional groups and differ only in length of hydrocarbon chain. The most common of Capsaicinoids compounds is ‘ Capsaicin’ which is the major constituent of chilli peppers and also responsible for their pungent taste. [3d chem.]

1. 2 Structure of Capsaicins:

Capsaicin figure 1. 2. 1

Dihydrocapsaicin figure 1. 2. 2

Nordihydrocapsaicin figure 1. 2. 3

Homocapsaicin figure 1. 2. 4

Homodihydrocapsaicin figure 1. 2. 5

Out of all the Capsaicins, the capsaicin and dihydrocapsaicin are the major constituents of Capsaicinoids (make up 80-90% of capsaicinoids).

1. 3 Cis-trans isomerism in Capsaicin [3dchem]

Capsaicin can exhibit cis-trans isomerism due to the presence of C= C bond. The double bond prevents the molecule to rotate freely internally, therefore, giving rise to stereo isomers.

Cis isomer of the capsaicin is less stable and has higher energy due to steric hindrance. As the methyl groups are in close proximity to each other it causes repulsion between them and hence making it a less stable arrangement due to this added strain.

Trans isomer on the contrary has methyl groups further apart and doesn't have any steric hindrance, making it a more stable/low energy arrangement. Therefore, the Capsaicin is always found in the Trans isomer.

Figure 1. 3. 1 [http://www. homesteadcollective. org/mpg/science/majorcrap5. shtml]

1. 4 Scoville Scale

The scale for measuring the extent of heat in chillies was first invented by an American Chemist Wilbur Lincoln Scoville in 1912. The test he devised is known as the ' Scoville Organoleptic test' in which he had a group of volunteers to taste the chillies on their own and later diluted them with sugar and water until they didn't have any burning sensation left. The resulting dilution factor was called the ' Scoville heat value' of the sample and a number was then assigned to each sample of chilli i. e. Scoville unit, to ascertain the amount of dilution a chilli needs before it's hot flavour dies away. [3d. chem]

Table 1. 4. 1 Scoville heat values for Capsaicinoids [g6csy. net]

Molecule

Strength /Scoville units

Capsaicin

16. 1 million

Dihydrocapsaicin

16. 1 million

Nordihydrocapsaicin

9. 3 million

Homocapsaicin

6. 9 million

Homodihydrocapsaicin

8. 1 million

The hottest capsaicin found is in the chilli known as ‘ Naga Jolokia’, grown in India and has Scoville strength of 855, 000-105, 0000 units. The Habanero (Mexican chilli) are the runners-up with Scoville rating range of 200, 000-300, 0000. g6csy. net

As mentioned earlier, in addition to Capsaicins being used as food additives, they have important medicinal benefits and are known as ‘ Phytochemicals’. [3dchem. com] Due to having pharmaceutical and antioxidant properties, it’s widely used in anti-flammatory creams and ointments and also used as a counter irritant in surgical dressings and medicines. Moreover, they are also being used in nutritional supplements for pain relief and Arthritis. [cals. ncsu. edu]

1. 5. Extraction Methods for Capsaicin

Different methods have been devised as a way of extracting capsaicin from chilies and sauces. The simplest technique is to dissolve chilies in a polar solvent and placing the mixture on hot plates for half an hour and then filtering the extract via simple/vacuum filtration and making up with Methanol (100 ml).

The other techniques more efficient for extraction are as follows:

1. 5. 1 Solid Phase Extraction or SPE

This technique is particularly useful in extracting capsaicin as it requires pre-treating the sample prior to analysis. This reduces the amount of unwanted components that may interfere with the analysis.

The extraction is completed in 4 steps:

Conditioning the cartridge: This involves activating the cartridge by passing the sample through it to achieve same conditions with in the cartridge (e. g. to achieve same pH, composition etc as the sample).

Retention: The sample is applied to the cartridge and either contaminants are retained and analyte is flushed through the cartridge. Other way used is holding the analyte with in the column and the unwanted components are passed through the column.

Rinsing: The cartridge is then rinsed with distilled water to wash off the impurities.

Elution: The last step is to elute the sample with appropriate solvent and the extract can then be used for analysis.

1. 5. 2 Reflux

As demonstrated in this project, this method involves refluxing the chilies in methanol for appropriate duration. The reflux time required can be optimized by refluxing samples for different durations to establish the optimum time required by the capsaicin to leach.

1. 5. 3 Ultrasonication

This method can be used for extracting the capsaicin from sauces or capsaicin based creams in short time. The samples are soaked in Ethanol and placed in the ultrasonic bath for half an hour at high temperature. The ultrasonic vibrations release the capsaicin from the samples.

1. 5. 4 Supercritical fluid extraction or SCFE

In this method, the extraction solvent used is a super critical fluid. A super critical fluid (SCF) is a compound above its critical temperature and pressure. Therefore, an SCF is neither a liquid nor a gas. Hence, a super critical fluid has properties similar to liquids i. e. dissolving compounds and also gas like

properties e. g. transportation. SCFE also minimizes the matrix components. Due to these capabilities, this method is more efficient and quicker than the other extraction methods. Carbon dioxide and water are the most commonly used SCF. This technique has a variety of applications in food, petrol, and pharmaceutical industries.

1. 6. Previous research on Capsaicin

Capsaicin has been widely studied and researched by various organisations and institutions but insufficient literature has been published with respect to the analysis of chilli peppers and sauces. In this section, three articles will be discussed as all three papers deal with the analysis of chillies and sauces relevant to this project.

The first literature[1] investigated the concentration of Capsaicin and Dihydrocapsaicin in the Habanero peppers using Super Critical Fluid Extraction method (SCFE). In addition to the analysis of Habanero whole peppers, different parts of chilli were also examined for their Capsaicin content. The Habanero peppers were obtained from two different locations: Cunningham Research station and Bailey Farm (located in North Carolina, US).

The peppers were cut into seeds and shells and prior to extraction, the samples from Bailey farms were prepared fresh, oven dried and freeze dried and samples from Cunningham station were prepared in oven and freeze dried states. The whole peppers and seeds/shells samples were extracted using three polar solvents i. e. Methanol, Acetone and Acetonitrile. The Method for SCFE is as follows as stated in the literature: “ Fresh, oven and

freeze dried preparations (0. 5g dry weight) were extracted using a biomass: solvent loading of 15% (w/v) based on the initial moisture of the pepper samples/parts. Sample and solvent mixtures were homogenised in 50ml conical glass tubes and placed in a shaking water bath (50°C).” The extracts (2ml each) were then filtered and stored at -20°C until the analysis.

The preliminary work suggested that 1hr is sufficient to get good yield of capsaicin. The extracts were then analysed using Reverse-phased HPLC with UV VIS Detector. The HPLC was equilibrated with capsaicin standards (10, 30, 50ppm). The mobile phase composition was isocratic at 60: 40 (Acetonitrile: Water with acetic acid (pH 3)).

The researchers of this project compared the capsaicin and Dihydrocapsaicin concentrations from both locations. The results showed that the Cunningham station’s peppers had higher concentration of capsaicin in comparison with the Bailey farm’s chillies but the Bailey Farm’s pepper had higher amount of Dihydrocapsaicin than the Cunningham’s. The results suggested that samples that were oven dried and extracted with Acetone gave maximum yields of the Capsaicinoids. This literature also suggested that regardless of the solvent type and preparation state used, seed has the highest amount of capsaicin.

The researchers suggested the reason for differences in capsaicin concentration was due to different environments the fruits are cultivated e. g. chemicals used, weather conditions etc.

The second literature[2] deals with the analysis of three Capsaicinoids i. e. levels of capsaicin, dihydrocapsaicin and nordihydrocapsaicin in different

chillies, sauces and arthritis creams via reverse phased HPLC. This research employed a solvent extraction technique which involved addition of ethanol (extraction solvent) to the samples of ground chillies, sauces and creams and placing the samples on hot plates for 30mins. After cooling and filtration, the extracts were transferred into flasks (100ml) and made up to the mark with Ethanol. 5ml was withdrawn from this sample and filtered again into a syringe filter cartridge (0. 45µm pore size). This aliquot was then used for the analysis. A 1000ppm standard stock solution was used to make standard capsaicin solutions ranging from 1-50ppm and ran through HPLC. The mobile phase in this research was made up of ACN, water and phosphoric acid (0. 1%). The UV detector was set at 280nm and 205nm to determine samples responses at different wavelengths. In addition, Isocratic and Gradient elution were used.

The findings from this journal suggested that Capsaicinoids present in very little concentrations (e. g. 0. 5ppm) were detected better at 205nm wavelength using gradient elution rather than Isocratic method. However, results also indicated that for analysis of Capsaicinoids present in greater concentrations, Isocratic elution and UV wavelength at 280nm. The concentrations of the Capsaicinoids were expressed in terms of the Scoville units and the value calculated for the Habanero peppers (150, 000) in this experiment was different to the literature value range (200, 000-300, 000). The researchers attributed this fact to variations in the environment e. g. weather etc.

The third literature[3] determined the capsaicin and dihydrocapsaicin content in chilli peppers. The chillies were grounded for 10mins and Acetonitrile

(30ml) was added to the crushed peppers and again grounded for 20mins. The solid residue was filtered and an aliquot (1ml) was made up to the mark with Acetonitrile. (in 10 ml flask).

The extraction method used in this work was Solid Phase Extraction (SPE): An SPE cartridge was conditioned with Acetonitrile, methanol and water and the capsaicin extract (10ml) was then applied to the cartridge and the analyte was eluted with methanol (4ml) and then again with 1 ml of methanol (containing 1% acetic acid).

The analytes were then run through reverse phase HPLC using UV-VIS detector (at 281nm); mobile phase consisted of 77: 33 (Methanol: Water). The HPLC was eluted first with the standards so as to obtain the calibration graphs. The Capsaicinoids concentration of different chillies was expressed in Scoville heat units (similar to literature 2). The results showed that Habanero is the hottest amongst all peppers that were analysed i. e. Scoville heat value of 276, 000 which corresponded to the literature value range. The least hot pepper was Jalapeno (41, 000 Scoville heat units).

1. 6. 1 Comparison of three papers:

The researches have used different techniques to extract the Capsaicinoids i. e. SCFE and SPE. However, the extraction technique used in this project was Reflux (for chillies) and Ultrasonication (for sauces). The extractions were successful and all samples in general were detected which indicates that more than one method can be employed as a way of extracting the Capsaicinoids from chillies and sauces. Various parameters were manipulated as part of method development in literature 2 e. g.

Isocratic/gradient elution and different wavelengths. Similarly in Literature 1 three preparation states and three solvents were used to determine what state/solvent gives maximum yield of Capsaicinoids. Literature 1 also suggested that the hottest part with in the chilli is the seeds, however, the findings of this project have shown that the Endocarp contains the highest amount of capsaicin and dihydrocapsaicin. This is indicative of the fact that the amount of Capsaicinoids can vary even with in different parts of chilli. However, all researches discussed as well as this project has used polar solvents to extract the analytes and the analytical technique used was reverse phase HPLC which indicates it's usefulness in the capsaicin analysis in particular.

Once an analyte of interest has been extracted, it can be analysed by a process called Chromatography.

1. 7 Chromatography

The word Chromatography originates from Greek; Chroma means colour and graphein implies to write. [Skoog West Analytical Chem 7th Edition page 646]

The history of this separative technique dates back to early twentieth century when it was developed by a Russian Botanist Mikhail Tswett in 1903[D kealy Instant notes]. He used this method for separation of various plant pigments and samples were passed through a calcium carbonate column. The separated analytes were identified as they left coloured bands on the column. [Skoog West Ana Chem 7th Page 646]. Since its invention by the Russian Scientist, this method has been modified and developed in many

forms to give quantitative (amount of the analyte present) and qualitative analysis (identification of the unknowns) of complex mixtures. [d. Kealey instant notes page 119].

Separation in Chromatography is achieved by passing the sample mixture through the stationary phase by continuous flow of a mobile phase. This process is known as Elution. Hence, the chromatographic separation depends on the differences in the distribution ratios of the sample components between the stationary and mobile phase. Therefore, this capability of an analyte to migrate at different rates in both phases gives separation over a period of time and distance travelled. [D Kealey page 120]

Kx= Cs/Cm, where kx is the equilibrium partition coefficient and Cs and Cm are molar concentrations of analyte in mobile and stationary phase.

There are two types of Chromatography techniques: Year 2 notes page 25-26

1. 7. 1 Planar Chromatography

In this method, the stationary phase is composed of a flat bed of material which is made up of an adsorbed layer distributed evenly over a sheet of glass, plastic or Aluminum (known as Thin Layer Chromatography or TLC)

Paper Chromatography is also another type of Planar Chromatography in which the stationary phase is a sheet of cellulose material.

1. 7. 2 Column Chromatography

In this method, the stationary phase is a glass or metal column on to which the stationary phase is tightly packed onto a column where separation takes

place. Examples of Column chromatography are Gas Chromatography, High Performance Liquid Chromatography etc.

1. 7. 3 Chromatogram

The plot of detector response Vs elution time is known as the Chromatogram. [Year 2 notes page 25].

Figure 1. 7. 3. 1 [http://www. clu-in. org/characterization/technologies/images/retention. gif]

1. 7. 4 Retention time

In the above figure is a typical chromatogram and term tr is the time taken by the analyte to elute the column, known as Retention time. [year 2 notes page 28]

1. 7. 4 Dead time [d Kealey page 121]

Indicated as tm in the fig is referred to the dead time this is defined as " the retention time required by the non retained species (i. e. mobile phase molecule) to pass through the column."

A good Chromatogram should have well defined peaks having correct shape and symmetry (i. e. Gaussian shape), eluted in reasonable retention time (tr not too long or too short) and should be separated from the extraneous peaks. [Year 2 notes page 29]

1. 8 Describing a Chromatogram

There are four parameters used in chromatography that evaluate the quality of a chromatogram. These are:

1. 8. 1 The Capacity Factor, K’

It is the amount of mobile phase required to elute a particular peak. The K’ is calculated for the first and the last peak. This factor is particularly useful when establishing the best mobile phase composition in the HPLC.

K’ can be calculated as following:

K’= (tr-tm)/tm where

tr is the retention time and tm is the dead time.

A Chromatogram having well separated peaks in good retention time will have K’ values between 2-8.

1. 8. 2 The Selectivity Factor, Î±

This is the ability of a system to separate two analytes (A and B) and is calculated by:

Î±= trb – tm/ tra- tm, where

trb and tra are the retention times of analytes A and B.

A system where peaks are clearly separated has a value of Î± > 1

1. 8. 3 The Resolution factor, Rs

This determines the ability of a system to resolve two peaks that elute very close to each other. And can be calculated by:

Rs = 2 (trb-tra)/Wa+ Wb where

tra and trb are retention times and Wa and Wb are the peak widths of analyte A and B.

The value of Rs > 1. 5 for a good quality chromatogram.

1. 8. 4 The Efficiency Factor [D kealey page 126-127]

When separation takes place in a column, the chromatographic separation can be evaluated by the resolution factor, Rs or the efficiency factor. The efficiency is defined as the number of theoretical plates in a column. This factor evaluates the extent of band broadening of the analyte peaks. Increasing the number of plates and reducing their heights gives better efficiency and vice versa. The plate height can be calculated using:

H= L/N where

L is the length of the column (in mm usually) and N is the number of plates.

The efficiency factor N is calculated by:

$N= 16 (tr/W)^2$ for a peak with a good baseline

$N= 5. 54 (tr/W1/2)^2$ for a peak with a poor baseline and W1/2 is the width at half the maximum height of the peak. (year 2 notes 42-43)

1. 8. 5 Band Broadening

As an analyte passes down a column, the peaks become shorter and broader due to various factors that cause band broadening. The Van Deemter Equation explains the reason for the band broadening:

H= A+ (B/u) + Cu where H is the plate height and u is the linear velocity of the mobile phase. Other variables in the equation are explained below:

A- Eddy Diffusion: As the mobile phase carries the sample components through the stationary phase, some components pass through the column in a straight line whilst other may that are retained longer by the stationary phase may deviate from the straight path and cause the peaks or bands to be broader. If evenly sized particles are used for packing the stationary phase, then the Eddy diffusion can be minimized.

B- Longitudinal Diffusion: If the mobile phase is travelling at low velocities, then the analyte will spend more time in the column as analytes diffuse into the mobile phase. This longitudinal diffusion contributes towards peak broadening and can be minimized by an increasing the flow rate of the mobile phase. The increased velocity will reduce the retention time resulting in decreased effects caused by this phenomenon. [Veronica HPlc page 17-19]

C-Mass transfer: As discussed earlier in this section, the separation depends on the ability of the analyte to distribute itself between the stationary and mobile phase. As the mobile phase is constantly flowing, the true equilibrium distribution of the analyte is never established. This leads to increased retention times and thus resulting in peak broadening. [d kealey page 124]

5. High Performance Liquid Chromatography or HPLC

HPLC is a form of liquid chromatography which provides both qualitative and quantitative information about complex mixture samples in short time. The stationary phase in this technique is made up of very small fine particles and

the sample is forced through the column by mobile phase solvents under high pressure, hence also bearing the name High Pressure Liquid Chromatography. [page 1 Veronica HPLC].

5. 1 Mobile phase in HPLC

The main requirement for this technique is that the analyte must be soluble in the mobile phase as the mobile phase carries the sample mixture through the column where separation takes place. Therefore, if the analyte interacts with the mobile strongly, it will elute the column faster, leading to shorter retention times. [page 66 Chromatographic separations]. The mobile phase can either be a single solvent or different solvents combinations may be used. After suitable mobile phase has been chosen, the system can be set at isocratic or gradient conditions. In Isocratic conditions, the chosen ratio of solvents remains constant throughout the analysis e. g. in this project Isocratic mobile phase used for analysis. In contrast, the gradient mobile phase can be changed over the period of time. [Year 2 notes].

1. 9 Stationary phase in HPLC

The stationary phase in HPLC consists of a solid made out of micro porous material packed into the metal column. Silicas or modified Silicas with nonpolar organic groups attached are commonly used as column packing material. Out of all stationary phases used in HPLC, Octadecyl silica known as ODS or C18 is most extensively used due to its ability to separate the analyte components with high, intermediate and low polarities. Other stationary phases used in HPLC are Aminopropyl, Nitile, Sulphonic acid, quaternary Amines etc [d. kealey 159-161]

Elution in HPLC is carried out by determining the extent of interactions of the analyte with the stationary and mobile phases. The degree of separation of the sample components depend on their migration rates and distribution ratios in both phases.

1. 9. 1 Normal and Reverse phase HPLC

In normal phase HPLC (adsorption chromatography), the stationary phase is more polar than the mobile phase which is weakly polar. The separations are based on the relative polarities of the sample components. For instance, if species A is more polar than the species B, A will have strong affinity for the stationary phase and will be held in the column longer. This will result in species A having long retention time compared to B. Thus, in normal phase HPLC, least polar analyte elutes first.

In Reverse phase HPLC (bonded phase chromatography), the stationary phase in non polar and the mobile phase solvents used are polar. This phase is governed by the hydrophilic and hydrophobic properties of the analytes. Most polar analyte elutes first and vice versa.

As Capsaicinoids are relatively non polar, therefore, if the mobile phase polarity in reverse phase is increased then the analyte will have stronger affinity for the stationary phase and therefore will spend more time in the column, leading to long retention times.

The mobile phase solvents used in Reverse phase HPLC are water or aqueous buffer with an organic solvent. The use of protons in mobile phase composition improves the peak shape and travels in column quickly.

[http://ionsource. com/tutorial/chromatography/rphplc. htm#Solvents]

1. 9. 2 HPLC Instrumentation

Figure 1. 9. 2. 1 [http://www. youtube. com/watch? v= I-CdTU5X4HA]

Pump: In HPLC solvent delivery system, the pump is used to deliver the mobile phase solvents to the column under high pressure. Most commonly used pumps are ‘ reciprocating pumps’. The pumps used should be free of corrosion, must supply an accurate and controlled flow rate and should be pulse free.

The HPLC solvents for mobile phase must be degassed to ensure they are pure and free of any contaminants. This is done by passing an inter gas through the solvent reservoir in vacuum degasser.

Injector: The sample is injected through syringe into the injector port. The injection system must not interrupt the flow of mobile phase and should deliver sample into the column in small volumes (5-500µL).

Column: Most commonly used HPLC column is 25cm in length, internal diameter of 4-6mm and particle size of 5µm.

1. 9. 3 Detectors in HPLC

When the sample components elute the column at different rates, they pass through the detector, and the information form the detector is then displayed in the form of a chromatogram.

1. 9. 4 UV-Vis Detector

The detector is set at a specific wavelength which will be absorbed by the analyte. The degree of absorbance of UV radiation by the analyte is proportional to its concentration. (Beer Lambert law)

1. 9. 5 Diode array Detector or DAD

The Diode Array detector is a type of UV Vis Detector [D Kealey page 162]. When the sample reaches the sensor cells, UV radiation is shone on the analyte. The light source mostly used is a Deuterium lamp. After light passes through the cell, it’s dispersed onto the photosensitive diodes via diffraction grating or quartz prism. Every diode in the array detects different wavelengths. The measure of differences in extent of absorbance at different wavelengths by the sample components results in their identification and also gives information about the concentration of the analytes. [http://www. chromatography-online. org/topics/diode/array. html]

Other detectors used in HPLC are Fluorescence, refractive index, electrochemical detectors etc. [d Kealey 163-165]

Figure 1. 9. 5. 1

Experimental Method

This project was performed in four different experiments. In all experiments, the methods for extracting the capsaicin from chilli peppers and sauces were the same i. e. reflux and Ultrasonication. However, different masses of chilli peppers, their individual parts were used and solvent volumes were taken in these experiments. (see results)

2. 1. 1 Preparation of chilli samples and extraction

Several chillies were cut into small pieces and weighed into a 250 mL round bottom flasks. Ethanol (75 ml) was then added to chillies. A condenser (fitted with rubber tubing to the water tap) was fixed to the round bottom flask and solution was placed on isomantle (set at 80°C) and refluxed in fume cupboard. After cooling off, extract was filtered into 100ml flask and made up to the mark with ethanol. A 5ml aliquot was withdrawn into 1. 8ml sample vial using a 0. 45µm syringe filter. These aliquots were then analysed through HPLC.

2. 1. 2 Preparation of chilli sauces and extraction

The hot sauces were prepared by dilution of sauce (2g) with ethanol (15ml) in beakers and solutions were places in ultrasonic bath (set at 60°C) for 30 minutes. The extracts were filtered in the same way as chillies i. e. using 0. 45µl syringe and transferred to 20ml volumetric flasks and bringing up to the mark with methanol.

The equipment used was white tiles, knife, weighing boat and weighing scale.

2. 1. 3 Preparation of glassware

In analytical experiments, it’s of prime importance to ensure the glassware used is clean. Therefore, the glassware used in this project was washed first with deionised water and then with the solvents used.

2. 1. 4 Preparation of standards for Calibration

The standard solutions were prepared from 200ppm stock solution directly into sample vials. Eppendorf pipette was used for accuracy and 0, 20, 40, 60, 80, 100 ppm standards were made with HPLC standard Methanol.

Concentration/ppm

Volume of 200ppm

Capsaicin/ Î¼L

Volume of methanol/Î¼L

0

0

1000

20

100

900

40

200

800

60

300

700

80

400

600

100

500

500

2. 2 HPLC

The HPLC used in the laboratory was Agilent 1100 Series

Column- ODS hypersil

UV VIS Detector- G1315B Diode array detector (set at 280nm)

Flow rate- 1. 3 ml/min

Windows XP CPU

Mobile phase- Isocratic at 65: 35 Acetonitrile (2% acetic acid): water

Particle size- 5µm, Column dimension- 250mm x 4. 6mm

2. 3 Determining Î» max for UV- Vis detector

The Î» max for the detector was determined by placing methanol blank in UV spectrometer (Perkin Elmer with lambda 40) to calibrate it. After calibration, a 100ppm capsaicin standard was placed in the spectrometer and a graph was obtained with the optimum wavelength (see Appendix 1). This was 280. 40nm and the HPLC detector was set at this wavelength.

2. 4 Determining optimum mobile phase composition (see Appendix 2)

The mobile phase was altered to different ratios to establish what ratio gives the best separation and reasonable retention times (less than 7 minutes). The 80: 20 ratio (MeCn: water) gave shorter retention time but the peaks were eluted closer to each other. The 70: 30 ratio showed good retention time but peaks were still closer to each other. 50: 50 ratio gave long retention time and 60: 40 ratio gave good separation but retention time was longer. Therefore ratio of 65: 35 was used as this gave the best retention time and separati