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Iontophoretic Trans-Dermal Drug Delivery Through Sweat Glands

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by

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ABSTRACT

Although an iontophoretic trans-dermal drug delivery is known as an effective means for drug transportation through the human skin, it is not widely used because of the various side effects that come to life due to a high applied voltage of up to 80V.

This study introduces an alternative means of drug transportation through the skin by means of sweat gland activation and reduction of an applied voltage to ensure that the iontophoresis is safe. The skin conductance studies performed on the pulmar area using 50mM of NaCl showed that the activation of sweat glands led to the increase of the skin conductance up to 8-10 times which enabled us to use a lower voltage of 2V in order to achieve noticeable results during the actual drug delivery experiment performed in the points of low ionic resistance that are located on a human biceps, also the application of Vaseline on the experimental surface does not allow the decrease of a skin conductance for as long as 11 hours which enables us to do the drug delivery over a long period of time. Finally, the drug delivery was performed and tested by means of HPLC method.

Chapter 1

Introduction

Transdermal drug delivery has been a century old discovery, nevertheless, the topic still attracts new and revolutionary ideas of researchers everywhere in hope to find the one transdermal method that would be efficient, practical, safe, and cheap. One of the leading transdermal drug delivery methods out on the market and still in intense research is iontophoresis. The technique provides a noninvasive method to administer a controlled amount of drugs through the skin by applying an electric current. To simplify its mechanism, "Iontophoresis is a process of transportation of ionic molecules into the tissues by passage of electric current through the electrolyte solution containing the ionic molecules using a suitable electrode polarity, a so called (Needle-less injection)"(Korula, p.1)

The biggest question is: "Why do we need an Iontophoretic Drug Delivery if we can use direct injections or oral pills?" Needle injection of drugs is one of the most invasive methods of drug administering, it damages the tissue which may not heal in case of patients with diabetes, and in case of careless injection a patient can die from administering tiny bubble of air into a vain, infection or inflammation, it also is painful if performed improperly, also it is very hard to control the amount of drug administered by an injection and can cause an overdose and in some cases death.

On the other hand oral administration of drugs is relatively painless but can cause gastrointestinal incompatibility for some patients, occurrence of unnecessary metabolisms, as well as overdose of drugs occurring due to lack of ability to control the amount of drugs taken orally (Secundum Artem, p.2).

Iontophoretic drug delivery was designed and created to control the amount of drugs delivered into a human body through variation of applied voltage, ionic current, drug concentration or ionic strength.

Iontophoresis is the only acceptable and safe choice of drug delivery on the burned skin to avoid an infection. It is used in the tooth extraction and treatment of infected tooth root canals. It also is the best choice for patients with stomach problems. Patients with diabetes suffer a great deal from injections because their wounds do not heal very well and can cause deadly infections and inflammations, which is why scientists started thinking about the Iontophoresis as the safest drug delivery method.

There are three main passages through the skin's barrier: the stratum conium, the sweat duct, and the hair follicles (Nair, p. 468).The first passage is used by the contemporary iontophoresis methods. Although these methods have their advantages, they are not entirely safe. Contemporary iontophoresis is carried out using high voltage of up to 80V in order to overcome the skin barrier that has a resistance of 5MΩ. Side-effects and reactions like itching, erythema, irritation, skin pigmentation, permanent skin diseases, vascular and non-vascular diseases often occur under the delivery area (Gazelius, pg.2)

An alternative method would be to avoid the passage across the stratum conium, by utilizing the porous components of the skin such as the sweat ducts and the hair follicles, where the resistance is much lower. The passage through hair follicles is not so convenient because the channels are very narrow and also hairs prevent the electrodes from sticking to the hand and provide a very poor adhesion which is why the trans-dermal iontophoresis is performed most of the time on the hairless skin.

Activating the porous component of the skin one can lower the voltage needed to overcome the resistance of the skin, and decrease the area of the electrodes increasing the convenience of the drug delivery process and eliminating all the side effects that can occur.

The areas of the sweat glands and the hair follicles is small comparing to the total area of the skin, which is why using these pathways for drug delivery had been ignored, until now (Barry p.101). The following work focuses on the efficiency of drug delivery through the sweat gland.

Chapter 2

Iontophoresis and Contemporary Iontophoretic Methods

Iontophoresis is a process which involves increased transport of solute molecules into a tissue using an electric current (Secundum Artem, V.10, #4, p.1). Iontophoresis is basically a method of making "needle-less injections". Transdermal administration is mostly used for drugs requiring a relatively small dosage. It can be passive or active. In passive drug delivery, the drug diffuses through the dead cells of the skin, the stratum conium. On the other hand the active delivery is related to the ionized source of energy provided to drive the drug through the skin and its pores. Sometimes certain chemicals are used to enhance the skin before the delivery. In iontophoresis, the external source of energy is a direct electrical current. Ions traverse the skin according to the "like charges repel each other and opposite charges attract" principle (Secundum Artem, V.10, #4, p.1).

In practice, a drug solution is placed on an active electrode and that electrode is placed somewhere on the hairless skin, the passive electrode is placed close to the active electrode which provides an effective ion attraction force, and when an electrical current is applied the ions start penetrating the skin. Contemporary iontophoretic devices utilize current of up to 16μA, and a DC voltage of up to 80V. Fig. 1 shows the method of trans-dermal drug delivery currently used all over the world (Subramony, p.27).

In the Fig. 1 you notice that the electrodes are located near each other and the drug is getting pushed into the skin which requires a huge voltage and electrical current.
The delivery system consists of an anode, a reservoir containing the drug, a counter electrode, and a battery as the electrical energy source (Fig. 1). Under the application of an electrical potential, drug ions (D+) move into the skin while biological anions such as Cl- migrate from the body into the donor compartment to maintain electrical neutrality (Subramony, p.27).

The problem with this approach is in the location of the electrodes. The electrodes are located close to each other which means that if the system delivers a certain amount of drug into the stratum corneum under the influence of 80V voltage, most of this drug drifts from the positive electrode to the negative electrode through the stratum corneum without flowing into the epidermis, dermis or sub dermis.

Some companies claim that their iontophoretic device can deliver up to 50mg of drug like lidocaine trans-dermaly into the body judging by the difference in weight of the drug solution after and before the delivery, but they do not know how much drug has actually been delivered into the blood, which makes their approach very imperfect.

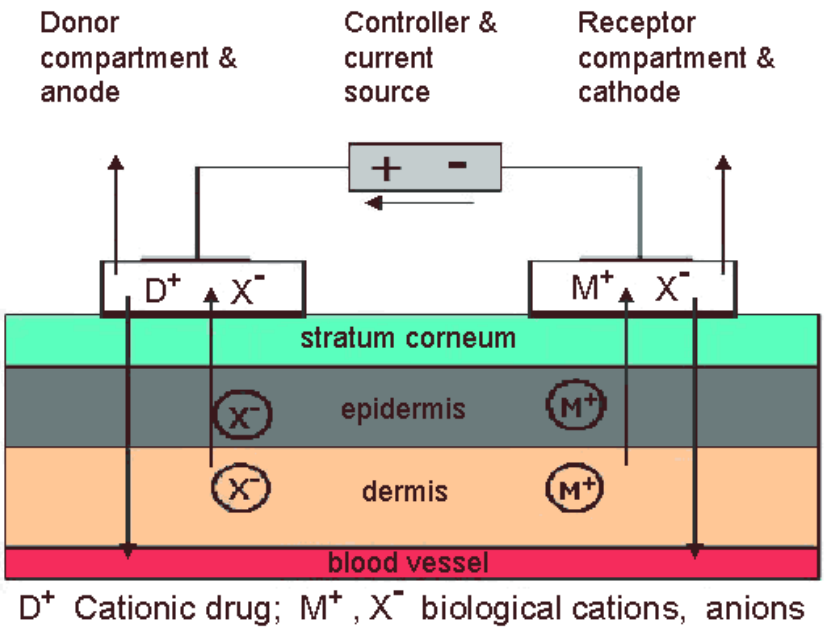


Figure 1 A contemporary iontophoretic drug delivery system (Subramony, p.27)

Chapter 3

Human Skin

Human skin is body's largest organ. In one square inch of human skin there are 19 million cells, 625 sweat glands, 90 oil glands, 65 hairs, 19 feet of blood vessels, and 19,000 sensory cells (Richardson, p.1). The outer layer, the epidermis, consists of rows of cells about 12 to 15 deep, and is between 0.07 and 0.12 millimeters thick (about as thick as a piece of paper). This top layer is composed mainly of dead cells that are being replaced constantly by newer cells (Richardson, p.1). Sometimes when the skin is intensely used the epidermis creates extra skin layers and makes the skin thicker which prevents injuries and skin damages, for example skin on the soles of feet of people who walk barefoot, and on the hands of farmers. The inner layer, or dermis, is a spongy, area that is about one millimeter thick, consisting mainly of collagen (a fibrous protein found in the skin) connective tissue (Richardson, p.1). The dermis is joined to the epidermis by nerves, blood vessels, hair follicles, sebaceous glands, and sweat glands. Sweat glands help to cool the skin and keep the body temperature constant (Richardson, p.1).

The sweat glands are distributed all over human skin, about two million of them all together, the purpose of which is to bring water to the surface of the skin where this water is vaporized and heat is in this manner withdrawn from the body (Asimov, p.258-259).

The water in this case is a sweat or perspiration. A sweat gland consists of a tiny coiled tube, the main body of which situated deep in the dermis. The tiny opening on the surface is a pore and is just barley visible to the naked eye. When you are working or playing hard, and heat production is increased, the sweat glands accelerate their production of perspiration which is also true when the temperature is unusually high. Perspiration then collects on the body in visible drops and we are conscious of sweating (Asimov, p. 265).

As you can see in the Fig. 2, the sweat gland and ductal structure arise in the sub dermal portion of the skin, beginning with the sweat gland and ending at the surface of the skin resulting in a pore. The gland itself consists of a tightly bound mass of tubule, which is supplied with nerve endings and capillary blood vessels (Sato, vol.20, #4). The nerves stimulate the gland to produce sweat in the event of a psychological stimulus or thermoregulatory process (Sato, vol.20, #4). The blood vessels, which surround the sweat gland and ductal portion in the dermis, supply the gland and duct with nutrients for functioning, and waste products for excretion (Sato, vol.20, #4). From the gland to the base of the epidermis, the duct remains relatively straight. The ductal walls are mostly a few cells thick in this region. These cells control absorption of sodium and other nutrients in the pre-sweat fluid before it travels to the surface of the skin (Fowles, p.362-378). The glands pump sodium, Na, into the lower portion of the duct, causing a concentration shift.

This shift draws more water into the duct, raising the level of water. Since the cells in the ductal wall remove sodium and other substances from the sweat, there is a different concentration gradient, that moves the solution up the duct, ultimately forcing it onto the surface of the skin (Fowles, p.362-378).

Before ending as a pore on the surface of the skin, the duct spirals throughout most layers of the epidermis and the entire stratum conium. This spiraling has been attributed to obtaining a better thermoregulatory efficiency by increasing the surface area that will absorb sweat and lose it to evaporation. This is what allows the body to cool itself in high temperatures (Fowles, p.362-378).

As water diffuses into the intercellular spaces, it forms narrow channels through which certain ions can migrate, allowing for an increase in conductivity through the skin when thoroughly saturated. The stratum conium cells are in contact with the solution in the duct and a solution that covers the surface of the skin during the experiment and the cells that border both regions of water are most likely to be affected first. The process continues until all cells are saturated, which leads to the closing of the pore which threatens the usefulness of our theory and must be overcome. The solution to this problem is given in the next chapter (Boucsein)

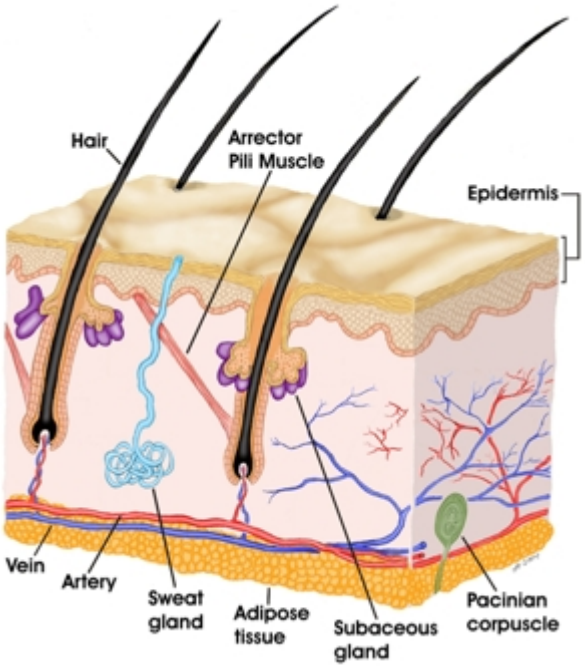


Figure 2. Human Skin (Sato, vol.20, #4)

Chapter 4

Method

It may seem that because the stratum corneum has a much larger surface area than sweat glands and hair follicles, it represents a very convenient pathway for drugs to flow from the surface of the skin into the blood vessels, but it also has an enormous resistance to anything that it comes across with, which is very important for an existence of a human body because the skin is our protective shield against water, toxic chemicals and environment. So in order to push drugs through our skin we have to utilize high voltage and current in our experiments which is very dangerous as was already pointed out in the previous chapters.

The method proposed in this work suggests the usage of the sweat glands as the only and the most effective passage of the drug into the blood vessels in spite of the fact that the sweat glands are very small and have very small surface area. Sweat glands are small, but as was mentioned before there are a lot of them (~625/inch^2), so by utilizing the sweat ducts, we can decrease the resistance of the skin and deliver a significant amount of drugs. We found that there are places on a human body where the resistance of the skin is very low, (about 500KΩ) and those are so called acupuncture points where lots of nerves come together, and those points are the best places for this experiment.

To be successful in this experiment the sweat glands must be open to create some passage ways. Sweat can be induced by increasing the body temperature by means of applying a heating pad to the delivery area, drinking hot tea/water/soup, or by attaching some external object like polyethylene to the skin, that way the temperature balance of the skin will be violated and the skin will start inducing sweat by itself gradually increasing its intensity in order to get rid of the attachment. The main problem related to the sweat glands that we had to overcome was the problem of the sweat gland closure due to the swelling of the dead cells of the stratum conium (Fowles p. 365). The sweat glands are prevented from closure by applying a thin layer of Vaseline on the surface of the skin in the delivery area. When the Vaseline is on the skin the stratum corneum is closed and so are all the pores of the skin including the sweat glands. When the experimental area starts sweating, the sweat glands start getting full of sweat and under a big pressure from inside the duct, the sweat opens holes in the layer of Vaseline and appears on the surface of the Vaseline. The sweat then makes its contact with a drug solution in the active electrode, but does not touch the skin because of the protective layer of Vaseline, and so this way Vaseline keeps the dead cells of the stratum corneum from swelling and closing the sweat pores and the sweat can be in contact with the drug solution for a long time until the Vaseline is stripped away.

By applying Vaseline we have managed to hold a high conductance reading for 11 hours, this is enough to be convinced that Vaseline partially solves the problems caused by pore closure.

Chapter 5

Experimental Set-Up for Skin Conductance Experiments

The systematic components of drugs delivery through sweat glands include DC power supply, 2 electrodes and an ionic solution Fig.3. Each component must be carefully chosen in order to provide an efficient and safe method.

A DC power supply of 2V applied to the delivery area gives an average of 4μA, which is much safer than 16μA current used in the current iontophoretic methods that utilize 80V. The power supply can be provided by a 2V battery for portable and practical usage, since the drug delivery period can last up to a few days.

The electrodes chosen for the current skin conductance study and the drug delivery are the TransQ-1GS electrodes, manufactured by Pro-Med. These Ag/AgCl electrodes stabilize the pH of the drug and prevent a shift of pH in a drug or in a tissue (Nair p. 470). Prevention of pH shift eliminates possible skin irritation, stabilizes drugs, and improves the drug delivery. Ag/AgCl electrodes are also nonpolarizing. This means that the skin and the sweat glands do not get polarized and so the development of a counter electromotive force (emf) can be prevented (Nair p. 4.70)). Also one of the most important advantages of Ag/AgCl electrode is that it has a very low junction potential and also it does not get oxidized and consequently does not enter the electrolyte.

The electrodes contain a Gel Sponge element which maximizes a uniform skin contact, and are the best for longer adhesion to prevent leakage of drug solution. The Gel Sponge also provides greater conductivity and consistent current and drug distribution. Also the gel makes sure that the drug solution does not make a direct contact with an electrode to prevent the electrolyze phenomenon. Fig. 4.

These electrodes provide an easy and practical way to introduce the drug solution into the electrodes. The larger area of the electrodes covers more area of sweat pores which maximize the total drug that can be delivered.

Active electrode on the bottom and Passive electrode on the top. Active electrode consists of adhesive tape, Ag/AgCl electrode, conductive gel, and a sponge. Passive electrode consists of conductive gel and Ag/AgCl electrode.

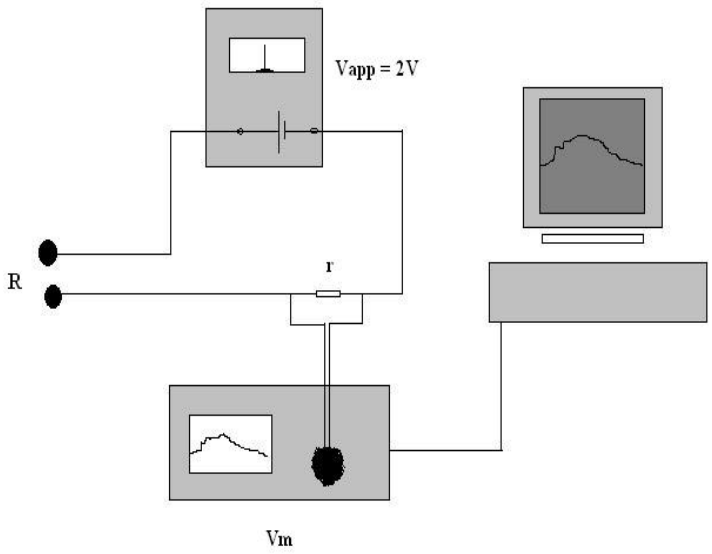


Figure 3. Schematic diagram of circuit and equipment used in the skin conductance study experiments.

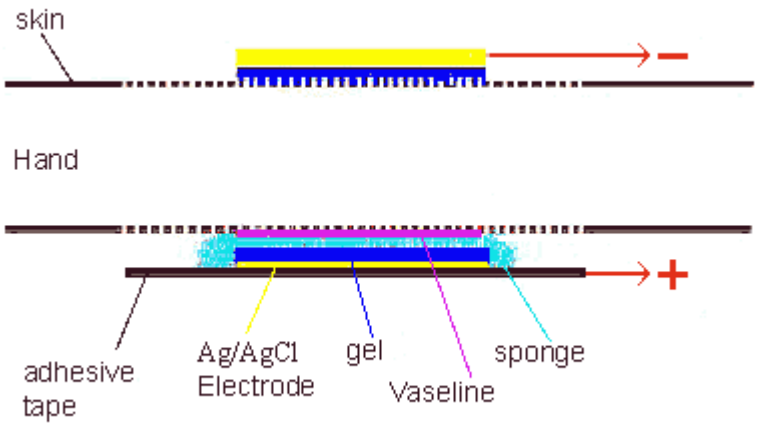


Figure 4. TransQ-1GS Ag/AgCl electrodes from Pro-Med.

Chapter 6

Skin Conductance Studies

As was said previously the schematics of the apparatus used in the skin conductance experiment is shown in Fig. 3. It consists of two Silver-Silver Chloride (Ag-AgCl) electrodes, a simple circuit with a data acquisition resistor, a voltage supply, an oscilloscope and a computer which records data. When the electrodes are attached to the skin, they form a resistor, the resistance of which is measured by placing it in series with a second (much smaller) resistor and connecting it to a voltage source. It is the voltage across the smaller resistor that is measured via the oscilloscope and recorded into a data file on the computer. From the data in this file, the measured voltages can be computed into conductance based on the simple relation:

$$G = \frac{V_m}{r(V_{app} - V_m)}$$

which is derived from the Ohm's law equations,

$$V_{app} = I(R + r)$$

$$V_m = Ir$$

$$G = 1/R$$

G - represents the conductance of the skin, **R –** is a resistance of the skin, **r –** is a data acquisition resistor, $\mathbf{V_{app}}$ - is the voltage applied to the circuit = 2 V, and $\mathbf{V_m}$ **–** is the voltage measured across the data acquisition resistor. During the process of sweating, the level of sweat in the duct increases and eventually reaches the surface of the skin, making a connection with NaCl solution and completing the circuit. Ones the connection is made, no further sweating should be necessary since the sweat in the duct that diffuses into the stratum conium is replaced by the unlimited supply provided by the electrode. Because of this, the ions can flow through the duct.

Factors that affect the drug permeation through stratum conium can be distinguished from a simple steady state flux equation for a given thickness of the skin (Eq. (1); Barry, 1983)

$$\Phi \sim CDK$$

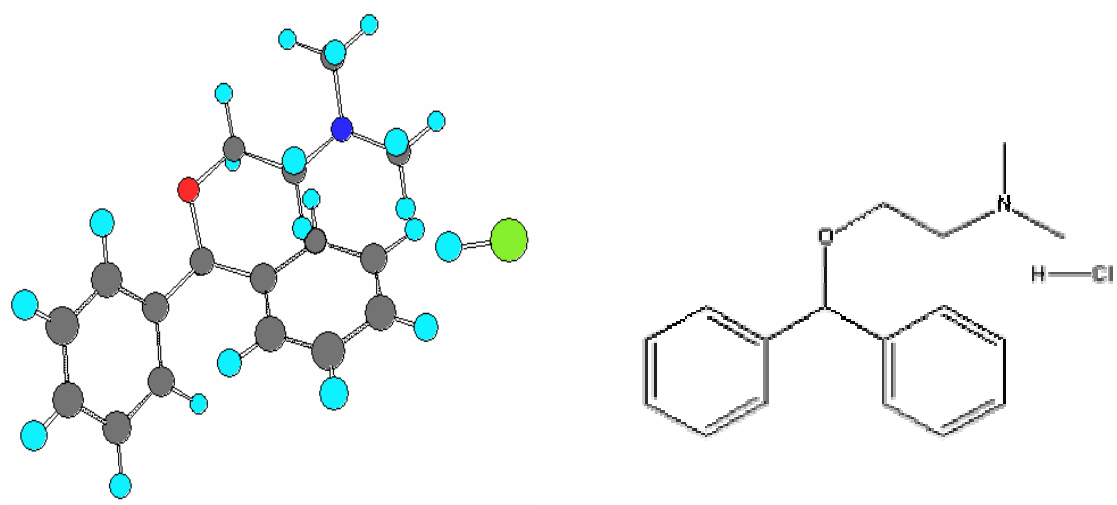
where $\Phi = dm/dt$ is a steady state flux of the drug, C is the drug concentration in donor solution, K is the partition coefficient of solute between a skin and the solution, D is the diffusion coefficient .

So factors that affect the drug delivery include the ionized state of the drug, and its pH range in which the trans-dermal permission of ionized state is maximum (Korula p.1). Other factors including good solubility in oil/water, low melting point (correlating with good water solubility), low molecular weight (less than 600 Daltons) (Barry, 1983) and a high concentration of the drug (Korula p. 2).

The drug solution chosen for delivery is Benadryl (Diphenhydramine Hydrochloride) which is a known antihistamine (see Fig.5).

This drug has been chosen for delivery because its solubility in water is very high (more than 10g in 100mL of water), its molecular weight is relatively low (~291.8 Daltons), and its melting point is also low (~166 C^o) (chemfinder.com).

The chemical formula of Benadryl is $(C_6H_5)_2CHOCH_2CH_2N(CH_3)_2HCl$ which is the same as $C_{17}H_{22}ClNO$. Benadryl can only be dissolved in water and it does not enter into a reaction. When dissolved in water, the neutral form of Benadryl becomes ionized so we observe the following: $C_{17}H_{22}ClNO + H_2O \Rightarrow R^+ + Cl^-$ which represents a complicated organic radical R with charge (+1) and a remaining non organic acid with charge (-1). This is clear because when salts of drugs are dissolved in aqueous solutions, ionized particles are formed and this process is called dissociation or ionization (Secundum Artem, V.10, #4, p.3). Now almost all the drugs are available in salt form because of great water solubility, and also the formation of ions is a typical behavior for all the water soluble drugs and chemicals which have at least one non organic ion, like salts of non organic acids which is the case we have. Under the influence of electric field of 2V these ions are transported into the skin through the sweat glands and driven into the blood stream. Benadryl can be detected easily by the blood or urine test about 8 hours of experiment and it has been delivered through the iontophoresis process by many others (Secundum Artem, V.10, #4, p.2).
Benadryl is also a non-prescription medication that relieves allergy symptoms, hypersensitive reactions, motion sickness, and uncontrollable muscle movements.



(a) (b)

Figure 5. The molecule of Benadryl $C_{17}H_{22}ClNO$ (a) in 3 dimension (www.chemistry.org) and (b) in 2 dimension (www.chemfinder.com)

Chapter 7

The Results of the Skin Conductance Studies

The results presented in this chapter are the averages of many similar experiments carried out at different times of the day and on different days. In order for an experiment to be successful, a subject has to sweat in the particular areas of the experiment where the electrodes are placed. So ones a subject sweats well enough to activate and open the sweat ducts, the potential readings increase significantly.

In order to make sure that the electric field goes through the sweat glands under the positive electrode to the negative electrode through the sweat glands located under it, both electrodes were placed facing each other, positive electrode on one side of a biceps and the negative electrode on the opposite site, so that the electric field goes straight from the positive to the negative electrode, which makes sure that the electric field is traveling through the sweat glands and not just through the skin (see Fig. 4).

The experiments with 50mM of NaCl showed that the conductance of the dry pulmar skin was about 7-8 times less than the conductance of the sweating pulmar skin, which means that the sweat glands contribute a lot to the conductance of the skin and the activated sweat glands increase the conductance enough to deliver drugs. The average results of the experiment carried out on a palm is presented in Fig. 6.

The "Control" value of the average skin potential is about 3mV and after 30 minutes it increases until about 5mV. This increase is due to sweating that is induced due to the violation of the temperature balance of the skin after attaching the electrodes to the skin and also due to the hydration of the skin when the electrolyte makes contact with the skin. As you can see the potential (therefore the conductance) increased tremendously (with respect to the "Control" value) in case of the sweating experimental area. Over the course of 10 minutes the conductance reached its peak value and stayed there for about 5 minutes, then decreased. The decrease of the conductance was due to the closure of the sweat glands. The "Sweating with the Vaseline on" experiments were carried out the same way as the previous ones, but here the experimental area was covered with Vaseline which prevented the closure of the pores. To prove that the pores remain open for a longer period we did 11 hour experiments on the biceps skin in the points of low ionic resistance (~0.5MΩ) and the conductance was still holding high. One of the results is shown in Fig. 7(a). It clearly shows that that Vaseline is very efficient in preventing pores closure and holding a high conductance.

Fig. 7(b). shows the same experiment but with Diphenhydramine HCl instead of the NaCl drug solution. As you can see from Fig. 6 it is clear that the conductance of a palmar skin increase about 7-8 times when we use NaCl, activate sweat glands and use Vaseline to eliminate the stratum corneum, and from Fig. 7(a) it is clear that by doin the same experiment in the points of low resistance on a biceps we get even bigger values for the conductance of the skin and again increase the conductance about 7-8 times.

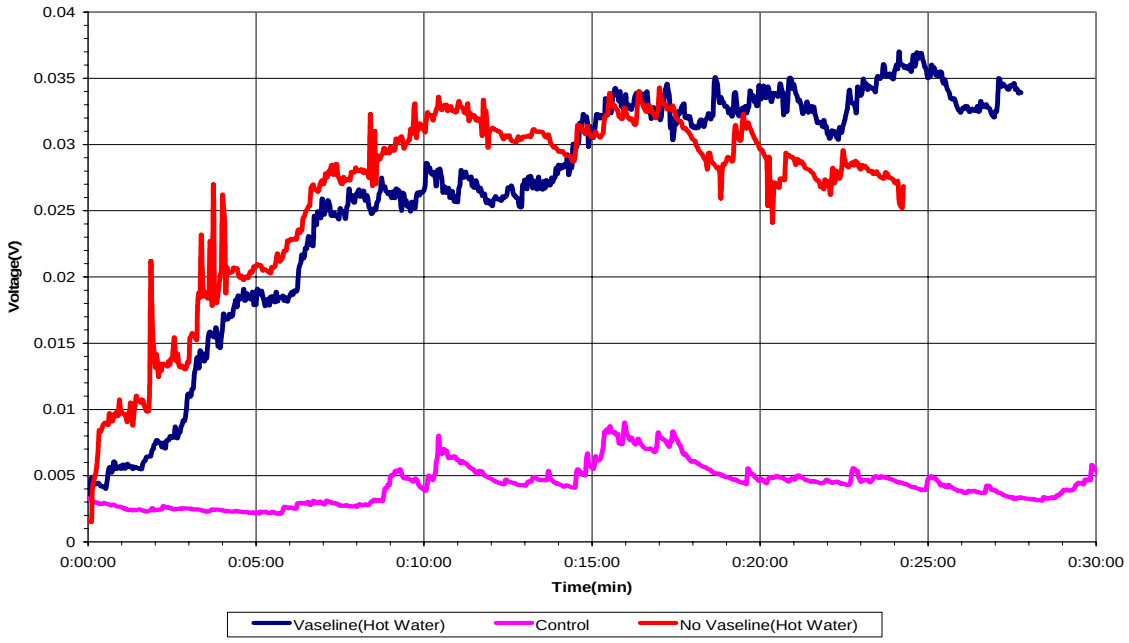
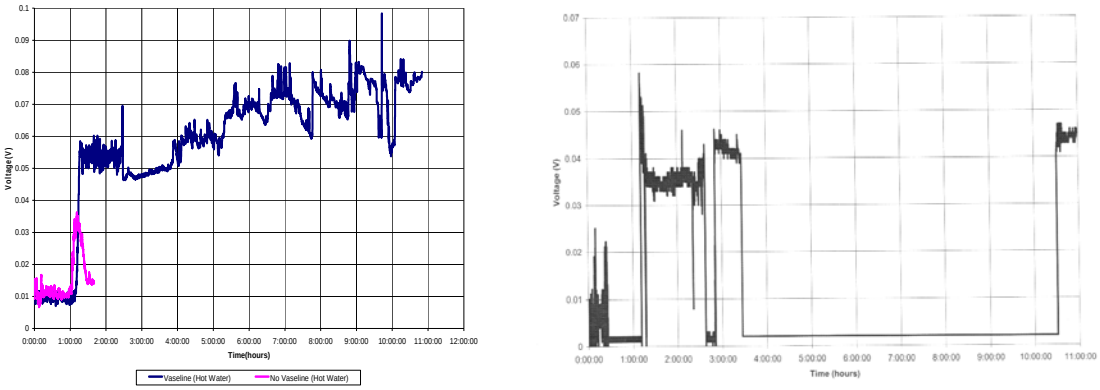


Figure 6. The average potential versus time graphs for the “Control” (dry skin-pink line), “Sweating”(red line) and “Sweating with the Vaseline on” (blue line) experiments carried out on a palm.



(a) (b)

Figure 7(a). "11 hour" experiment on the biceps with Vaseline (blue line) and about 2 hours experiment without Vaseline (pink line), (b) 11 hour experiment on the biceps with Vaseline using Diphenhydramine HCl.

Chapter 8

High Performance Liquid Chromatograph (HPLC) as a Separator

The purpose of this chapter is to introduce the theoretical aspects of chromatography. In chromatography, a liquid sample is applied to a narrow cross section of a column which is filled with a sand-like Silica based material composed primarily of Carbon chains . A solvent is pumped through the column, sweeping the sample from the inlet to the outlet and through a detector. It is the differential interaction of the various components in the sample with the stationary phase of the packing material which effects the separation. This is shown in Fig. 8, where a mixture of the molecules, A and C, have been injected into a narrow zone at the inlet of the column (Beckman, p.1). While moving with the solvent (mobile phase), A and C become slightly separated because C shows a greater affinity for the stationary phase and moves more slowly than A. Eventually A elutes, distinct from and followed by C - this separation is the goal of the chromatography (Beckman, p.1). A chromatogram is a two dimensional plot of molecules eluting from a column; measurement of absorbance or some other defining property is plotted against elution time or volume. In HPLC, the mobile phase is a liquid and the stationary phase is usually a coating bonded to a small solid particle, called a support, which is contained in a metal cylinder or column. A molecule is retained if it partitions into the stationary phase rather than merely traveling with the mobile phase (Beckman, p.1).

There are two kinds of chromatography: “Reversed Phase Chromatography (RPC)” and “Normal Phase Chromatography (NPC)”. In reverse phase chromatography, the packing is nonpolar and the solvent is polar with respect to the sample. Retention is the result of the interaction of the nonpolar components of the solutes and the nonpolar stationary phase. On the other hand in the normal phase chromatography, the packing is polar and the solvent is nonpolar with respect to the sample.

Typical stationary phases are nonpolar hydrocarbons, waxy liquids or bonded hydrocarbons (such as C_{18}, C_8, C_4, etc.) and the solvents are polar aqueous-organic mixtures such as methanol-water, acetonitrile-water, or phosphoric acid-water (Beckman, p.1). The C_{18}, C_8, and phenyl bonded phases are most often used in the reverse phase mode and it has been estimated that 60-90% of all analytical LC separations are done on bonded phases in the reverse phase mode (Beckman, p.1). Bonded phases made by covalently bonding a molecule onto a solid stationary phase are intended to prepare "liquid coatings" which will be permanent. Silica is a reactive substrate to which various functionalities can be attached or bonded. The functionalities most widely bonded to silica are the alkyl (C_{18} and C_8), aromatic phenyl, and cyano and amino groups (Beckman, p.1).

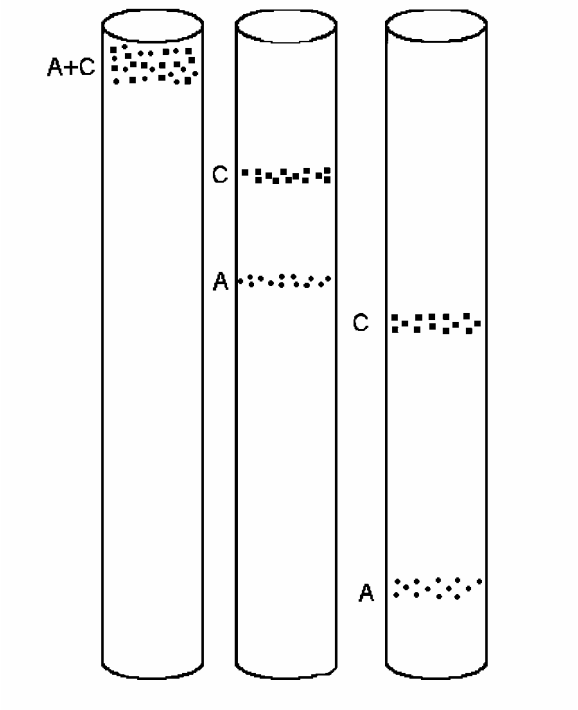


Figure 8. Chromatographic Process

8.1. Instrumentation

Fig. 9 presents a block diagram showing the components of an HPLC instrument. Solvents are pumped into a Solvent Delivery System where they are mixed together and passed through a Degasser and Debubbler to get rid of gasses and bubbles inside of the solvents. After that, the mixture of solvents is pumped to the injection port where the sample is injected into the (HPLC) instrument. The solvents mix with the sample and the whole solution is passed through the column. In the column the chemicals are retained and one after another they flow to the detector which is a UV-Vis Spectrophotometer most of the time. UV-Vis provides the peaks as the signals received from each individual chemical.

8.2. HPLC Analysis

The first and the most important part in HPLC analysis is the development of a method by which the sample is going to be analyzed. The method contains such important information as the flow rate of the solution through the column, the wavelength of detection of a specific chemical of interest, the temperature of the environment inside the instrument, the solvents that are used and their proportion at each moment of time during the experiment, the duration of the whole experiment, the name of the chemical of interest, and many more parameters which have to be set before starting the experiment itself. Each and every parameter mentioned above can change the result dramatically. When the peak retention time of the pure chemical of interest is found, the method can be considered successful and the real experiment can be done.

After finding the method the calibration should be performed and a calibration curve should be obtained in order to determine the amount of the chemical of interest in a sample. After this is all done, the sample should pass through the Solid Phase Extraction (SPE) process. Here the sample is passed through a special pre-treated and moisturized cartridges that contain material similar to the bedding material of the column itself. This process eliminates most of the contaminants and big particles in the sample so that it is ready to be injected into the HPLC instrument.

When the final chromatogram is on the screen, the peak of the chemical of interest is already visualized among the rest of the peaks in the sample and the peak area is presented. This peak area is the key to find the amount of a specific chemical in the sample.

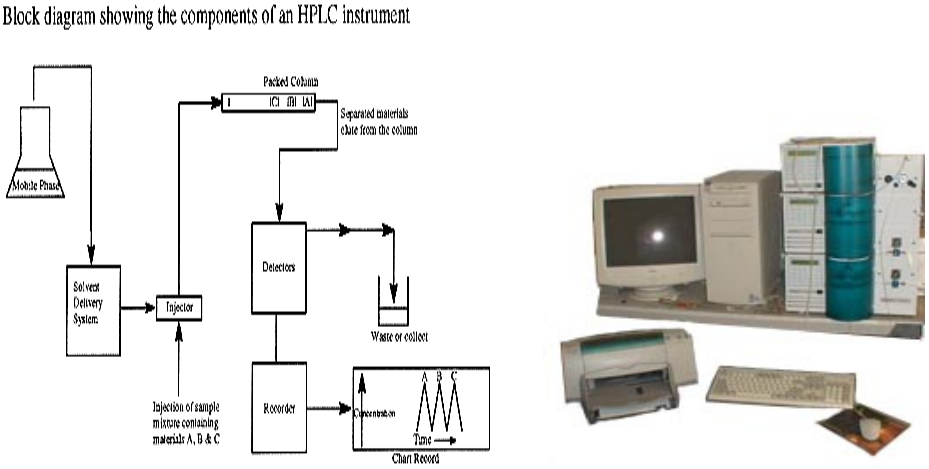


Figure 9. The components of HPLC instrument and the instrument itself.

Chapter 9

The Results of the Drug Delivery Experiment

Finally we performed a trans-dermal drug delivery on the biceps skin of a subject for 8 hours and collected the urine to determine the amount of Diphenhydramine Hydrochloride in it. That urine was then worked up under the solid phase extraction process. SPE process eliminates all kinds of contaminants and big particles from the urine so that they don't prevent us from getting the final urine analysis by means of a special **H**igh **P**erformance **L**iquid **C**hromatograph. The HPLC method that has been used to separate the Diphenhydramine Hydrochloride was carefully chosen to get the best separation.

Column: ZORBAX Rx/SB-C8 (4.6 x 150 mm), Solvent A: 20mM of KH_2PO_4 Phosphoric Acid, Solvent B: 10% Acetonitrile (60/40), Flow rate: 2mL/min, Temperature: 25^{o}C, Detector: UV at λ = 254 nm.

Fig. 10 presents the change of solvents A and B during the whole separation process that leaded to the best separation of Diphenhydramine HCl peak from the rest of the chemicals in the urine. The first experiment was done just on the pure sample of 1mg of Diphenhydramine HCl in 100mL of HPLC grade water to find out the retention time of the drug. The result is presented in Fig. 11. This chromatogram shows us that the retention time of Diphenhydramine Hydrochloride is t_R=3.655 ± 0.02 minutes. After that we performed 5 consequent experiments at 10μL, 8μL, 6μL, 4μL, and 2μL injection volumes for calibration purposes.

The constructed calibration curve is presented on the Fig. 12. Each experiment was repeated a few times to determine the measurement errors which you can see on the curve. The X and Y errors are presented as bars around the points.

After the calibration curve was constructed we performed the drug delivery on the same subject for 8 hours. After the urine was collected it was passed through the solid phase extraction process and 5μL of it was passed though the HPLC instrument using the same method mentioned above. The result is shown in the Fig. 13.

On Fig. 13 you can clearly see the peak of Diphenhydramine HCl having an area of 0.255 Volts*sec. This point is represented on the calibration curve as a big square having a peak size of 0.255 V*sec and the amount of 2.92μL. Consequently for 1μL injection volume we will get a peak having an area of 0.055 V*sec and the amount of 0.584μL.

This point is presented on the calibration curve as a big triangle.

Each round point on the calibration curve represent experimental data obtained from HPLC instrument after analysis of 1mg of pure Diphenhydramine HCl in the 100mL of HPLC grade water along with the measurement errors. The big square represents the Diphenhydramine HCl peak with its parameters (amount and peak size), obtained from 5μL urine injection volume after the drug delivery. The big triangle represents the same but from 1μL urine injection volume. Also you can see the R^2 coefficient and the calibration function.

We also have performed an analysis of a regular urine without Diphenhydramine Hydrochloride inside to determine whether the found peak is the peak that we are looking for, or a regular fluctuation. As you can see from the Fig. 14, we found no evidence of any sort of Diphenhydramine peak which shows that our results are very reliable because the area under the Diphenhydramine peak is much more than the measurement error which is about 4% .

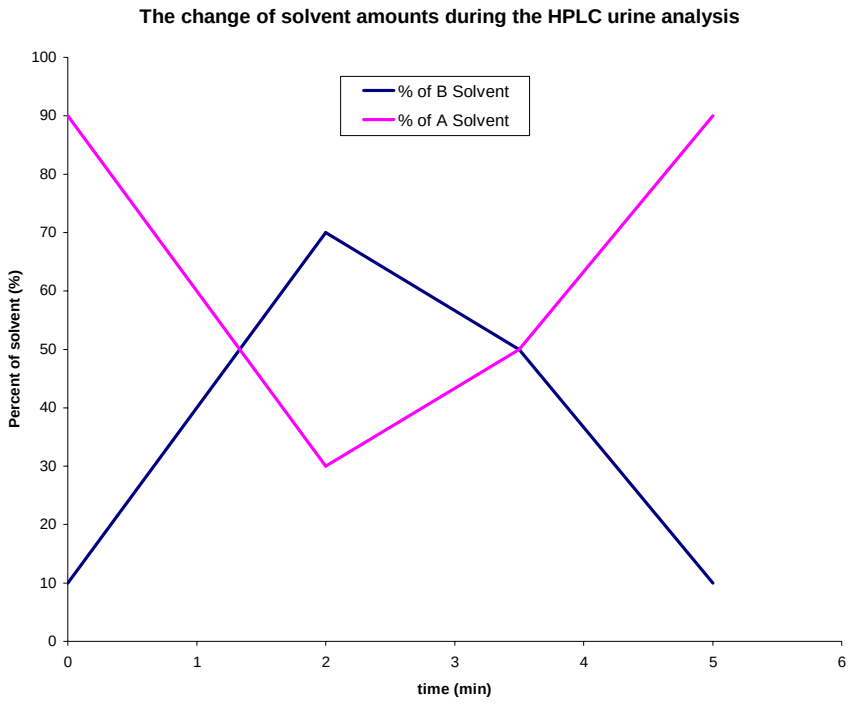


Figure 10 Change of the amount of solvents A and B during the whole urine analysis.
Solvent A: 20mM of KH_2PO_4 Phosphoric Acid.
Solvent B: 10% Acetonitrile in the HPLC grade water.

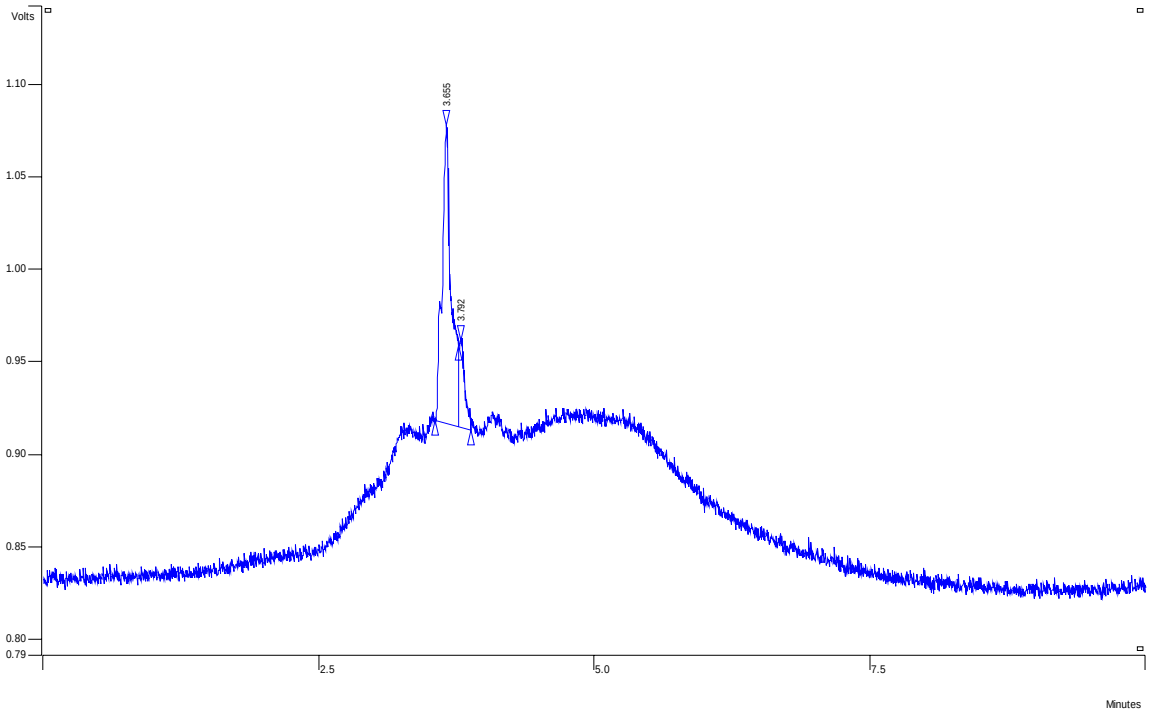


Figure 11 . The chromatogram of 1mg of pure Diphenhydramine Hydrochloride in 100mL of HPLC grade water.

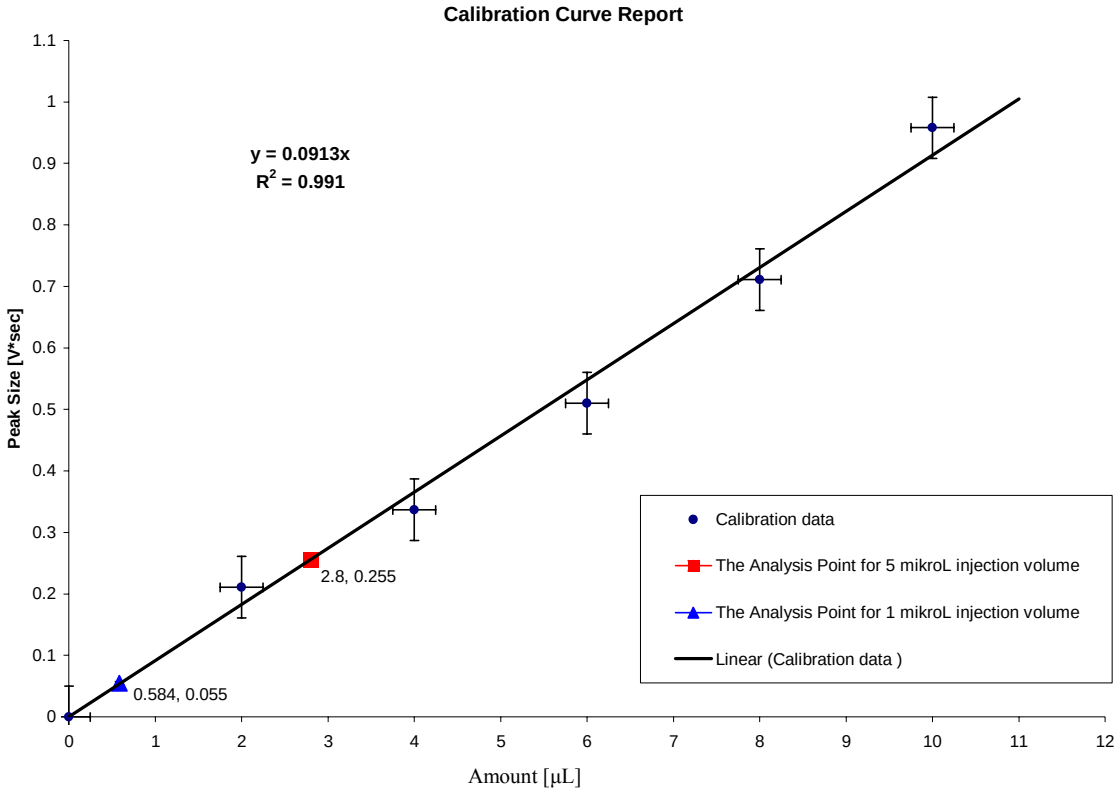


Figure 12. Calibration curve report.

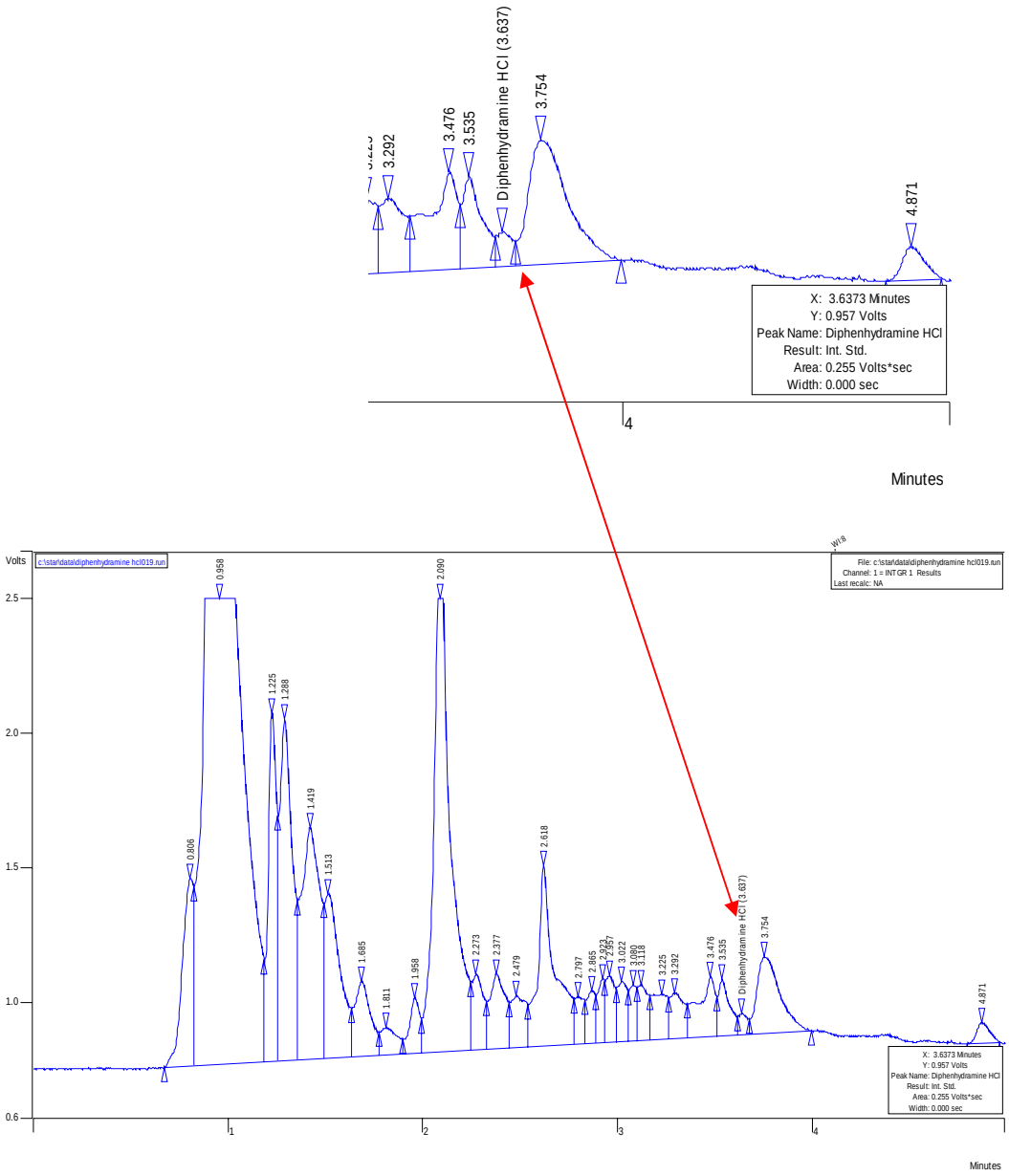


Figure 13. A chromatogram from urine analysis of a subject who was under 8 hour drug delivery experiment.

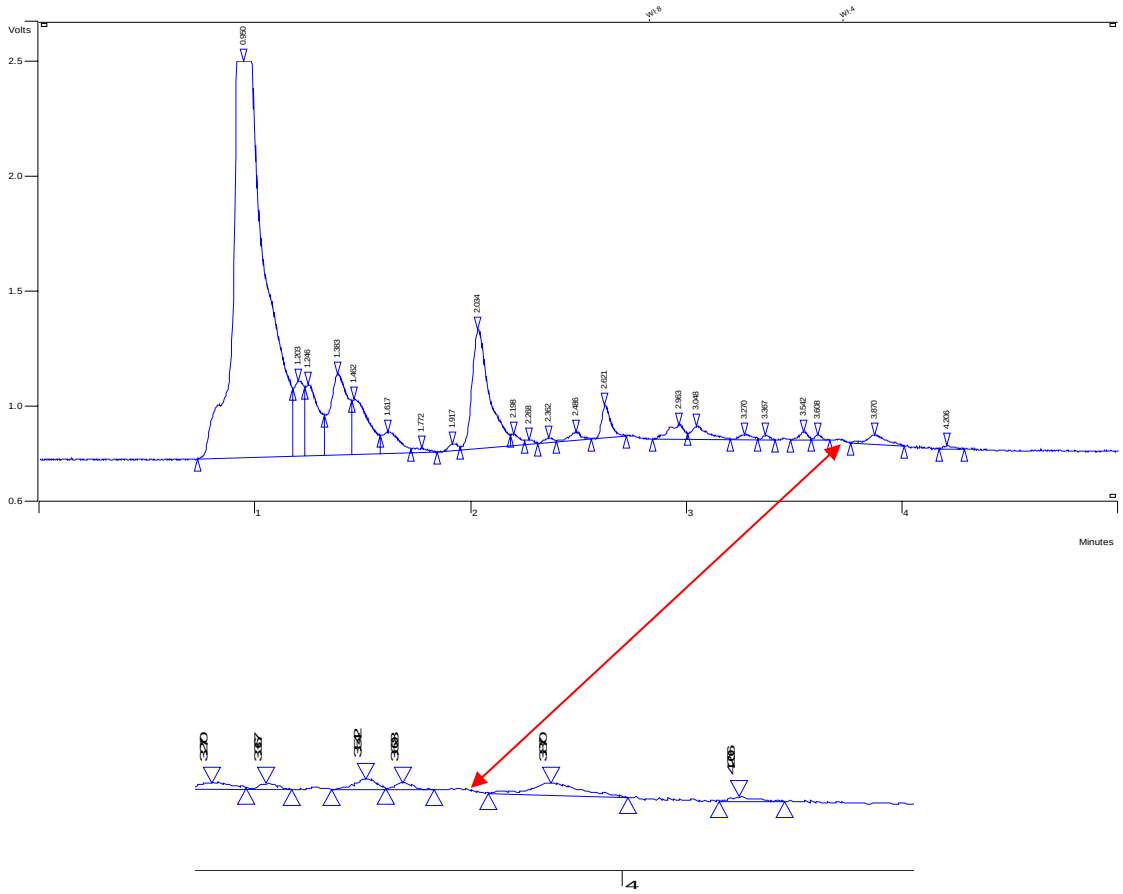


Figure 14 The HPLC analysis of pure urine without Benadryl.

9.1. Results

The data analysis shows that we found about 6 ± 0.24ng of Diphenhydramine Hydrochloride in 1μL of injected urine that was obtained from 8 hours of experiment. The average amount of urine after the experiment is about 300mL so we find about 1.8mg of Diphenhydramine Hydrochloride in the urine after 8 hours of experiment. All of these experiments were reproduced taking into account the measurement errors. The drug delivery experiments without Vaseline showed about 3 times smaller results, and the diffusion experiments without Vaseline and electric field showed about 4 times smaller results.

As specified in (WebMD Health Source) Diphenhydramine is widely distributed throughout the body, including the CNS. Little amount, is excreted unchanged in the urine and most appears as the degradation products of metabolic transformation in the liver, which are almost completely excreted within 24 hours, and according to this, there is a lot more Diphenhydramine Hydrochloride in the whole body than is found in the urine. When a drug is taken orally it passes through lots of organs before it reaches the blood vessels and so it is lost in the digestion process, and many other places. This means that only some small amount of drug goes into the blood and even smaller amount goes into the urine. As indicated in the (Ohtsuji M.) the maximum amount of drug found in the urine is about 2% of the drug amount taken orally and is about 1% of the drug amount taken through the diffusion processes (trans-dermal patch or cream) because most of the drug is broken down and lost in the process of getting into the blood and even after that before it gets into the urine.

On the other hand I.V. and iontophoresis are more efficient ways of drug administrations because most of the drug goes into the blood right away and that is why the oral does is twice as much as the I.V. or iontophoresis dose. And so it was experimentally established and confirmed that only about 4% of Benadryl taken by an I.V. injection or iontophoresis is found in the urine (Chaya Venkat). That means that we deliver about 45mg of Benadryl in 8 hours of drug delivery. The maximum Diphenhydramine HCl injection dose for adults is 75mg/day which means that we can deliver this dose by doing 13 hour experiment. The minimum injection dose for adults is 12.5mg/day, and we can deliver that in only 2 hours. For children the maximum injection dose is about 37.5mg which we can deliver in 6.5 hours and minimum injection dose is about 6.25mg which we can deliver in just 1 hour of drug delivery.

9.2. Conclusion

The new method of the sweat gland activation enables us to overcome the skin resistance. The swelling of the stratum cornium is prevented by applying a thin layer of thick Vaseline to the skin. We can deliver the minimum Benadryl injection dose in 2 hours for adults and 1 hour for children (6-12 years old) and we can deliver the maximum Benadryl dose in 13 hours for adults and twice faster for children. Our drug delivery method is much safer than the methods that are used all over the world, which is why this method provides a foundation to deliver drugs more safely and efficiently without any possible side effects.

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