CHAPTER II

REVIEWS OF RELATED LITERATURE AND RESEARCH

Skin whitening agent

Hyperpigmentation is the result of an increased amount of melanin in the epidermis, the dermis, or both. This pigmentary change can be divided into 2 pathophysiologic processes: melanocytosis (increased number of melanocytes) and melanosis (increased amount of melanin). Depigmenting agents work best when melanosis or melanocytosis is restricted to the epidermis. Depigmenting agents can be divided into several groups [7]:

Phenolic compounds include the following:

1. Hydroquinone
2. Monobenzylether of hydroquinone
3. 4-Methoxyphenol
4. 4-Isopropylcatechol
5. *N*-acetyl-4-S-cysteaminylphenol

Nonphenolic compounds include the following:

1. Corticosteroids
2. Retinoic acid
3. Azelaic acid
4. *N*-acetylcystein
5. L-ascorbyl-2-phosphate
6. Kojic acid

Hydroquinone, a skin-bleaching agent used to lighten areas of darkened skin, is a hydroxylphenolic chemical compound with the chemical name 1, 4-benzenediol that is a type of phenol. In human medicine, hydroquinone is used as a topical application in skin whitening to reduce the color of skin. It main use is as a depigmentation ingredient to fade melasma (darkened areas) and age spots on the skin and to brighten the complexion, which gives the skin its pigmentation, by inhibiting an enzyme called tyrosinase. It is also found in other cosmetics, such as hair dyes and

products for coating finger nails. At the moment, this policy is under review by the FDA. Products based on hydroquinone in concentrations of 4% or more can only be purchased with a doctor's prescription. In some cases, doctors may prescribe preparations containing up to 10% hydroquinone, and sometimes more [8].

Retinoic acid (tretinoin), a nature occurring metabolite of vitamin A (retinol), was the first synthesized retinoid that mediates the functions of vitamin A required for growth and development. Currently, retinoic acid is a designated as a prohibited substance for face cosmetics. It can only be purchased with a physician's prescription and is marketed in three concentrations of 0.025%, 0.05% and 0.1%. The treatment is tailored to each individual patient, according to age, skin type, history of sun exposure, and possible sensitivities to specific medications. Treatment should be initiated with the lowest concentration, and increased gradually as necessary. It was originally intended for acne treatment. Dermatologists observed its beneficial effect on the skin when treating adult women with acne. Retinoic acid lessens the consequences of photoaging. In addition, there are some beneficial effects of retinoic acid on chronological skin aging. It enhances cell division in the epidermis, replacing damaged and unorganized cells with new, organized cells. It also reduces melanin production. In the dermis, new collagen and elastic fibers are formed [8].

Corticosteroids are synthetic (artificial) version of hormones that are made by the adrenal gland. Hormones are powerful chemicals that have a wide range of effects on the functions of the body. The main purpose of corticosteroids is to reduce skin inflammation and irritation. Corticosteroids may be given orally, or injected into a muscle or a vein. In addition, corticosteroid preparations can be applied to an affected area of skin. As stated, dermatologists have available a wide range of preparations that contain corticosteroids of varying degrees of potency, which can be selected depending on the intensity of the skin inflammation. They may be given orally, or injected into a muscle or a vein. In addition, corticosteroid preparations can be applied to an affected area of skin. As stated, dermatologists have available a wide range of preparations that contain corticosteroids of varying degrees of potency, which can be selected depending on the intensity of the skin inflammation. The preparation used in dermatology in a concentration of 0.025-0.5%. Currently, corticosteroids are designated as a prohibited substance for face cosmetics [8].

Physical and chemical properties of hydroquinone, retinoic acid and corticosteroids

The structure, physical and chemical properties of hydroquinone, retinoic acid and corticosteroids are shown in Figure 2 and Table 2 [9, 10].

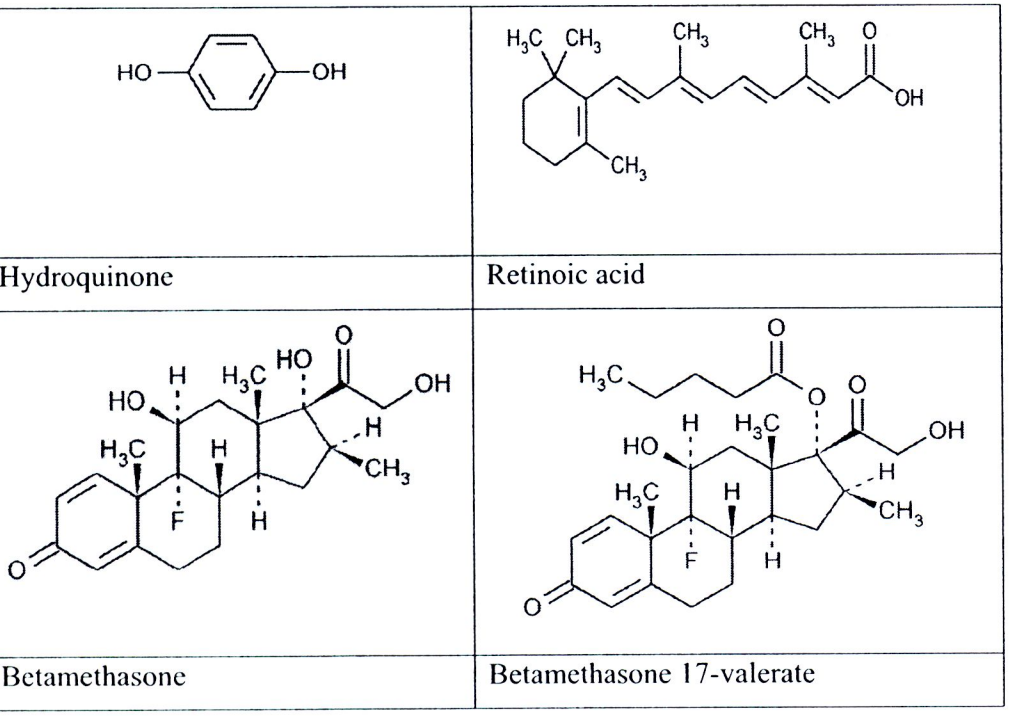


Figure 2 The molecular structure of hydroquinone, retinoic acid and corticosteroids

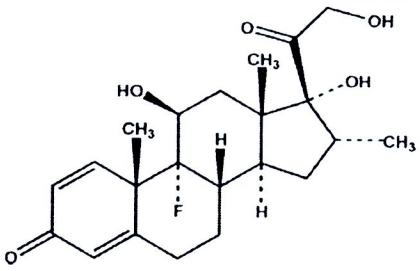
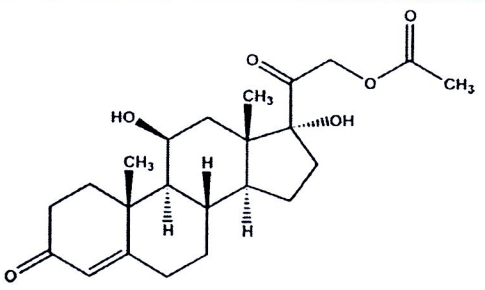
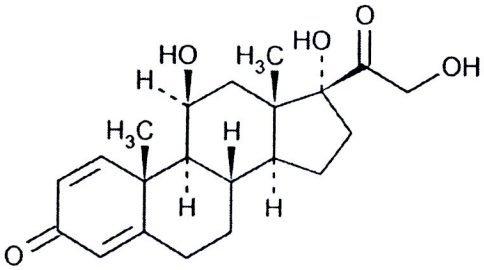
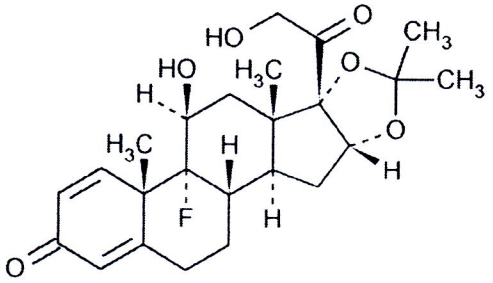
	
Dexamethasone	Hydrocortisone acetate
	
Prednisolone	Triamcinolone acetonide

Figure 2 (Cont.)

Source: Budavari, S., 1996 and USP 31, 2008

Table 2 The physical and chemical properties of hydroquinone, retinoic acid and corticosteroids

Compounds	IUPAC name	Molecular Formula	MW	CAS Number	Appearance and Odor	MP (°C)	BP (°C)	Density (g/cm^3)	Solubility
Hydroquinone	Benzene-1, 4-diol	$C_6H_6O_2$	110.11	123-31-9	Tan, gray, colorless or white needle crystals; odorless	172-174	285-287	1.332 at 15°C	Soluble in water, alcohol, ether; slightly soluble in benzene
Retinoic acid	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	$C_{20}H_{28}O_2$	300.44	302-79-4	Yellow to light-orange, crystalline powder; characteristic floral odor	180-182	-	-	Soluble in methylene chloride; slightly soluble in alcohol and in chloroform

Table 2 (Cont.)

Compounds	IUPAC name	Molecular Formula	MW	CAS Number	Appearance and Odor	MP (°C)	BP (°C)	Density (g/cm^3)	Solubility
Betamethasone	(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-9-fluoro- 11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one	$C_{22}H_{29}FO_5$	392.47	378-44-9	White to practically white crystalline powder; odorless	231-234	-	-	Sparingly soluble in acetone, alcohol, dioxane, methanol; very slightly soluble in chloroform and ether

Table 2 (Cont.)

Compounds	IUPAC name	Molecular Formula	MW	CAS Number	Appearance and Odor	MP (°C)	BP (°C)	Density (g/cm^3)	Solubility
Betamethasone-17 valerate	[(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-9-fluoro-11-hydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[*a*]phenanthren-17-yl] pentanoate	$C_{27}H_{37}FO_6$	476.59	2152-44-5	White to practically white powder; odorless	183-184	-	-	Soluble in alcohol; slightly soluble in benzene, ether

Table 2 (Cont.)

Compounds	IUPAC name	Molecular Formula	MW	CAS Number	Appearance and Odor	MP (°C)	BP (°C)	Density (g/cm^3)	Solubility
Dexamethasone	(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9- Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one	$C_{22}H_{29}FO_5$	392.47	50-02-2	White to practically white crystalline powder; odorless	262-264	-	-	Sparingly soluble in alcohol, acetone, dioxane, methanol; very slightly soluble in ether

Table 2 (Cont.)

Compounds	IUPAC name	Molecular Formula	MW	CAS Number	Appearance and Odor	MP (°C)	BP (°C)	Density (g/cm^3)	Solubility
Hydrocortisone acetate	[2-[(8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-10,13-dimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthren-17-yl]-2-oxoethyl] acetate	$C_{23}H_{32}O_6$	404.50	50-03-3	White or practically white crystalline powder; odorless	225	-	1.289 at 20°C	Slightly soluble in alcohol and in chloroform; very soluble in dimethylformamide
Prednisolone	(11β)-11,17,21-trihydroxypregna-1,4-diene-3,20-dione	$C_{21}H_{28}O_5$	360.45	50-24-8	White to off-white crystalline powder; odorless	235	-	-	Very slightly soluble in water

Table 2 (Cont.)

Compounds	IUPAC name	Molecular Formula	MW	CAS Number	Appearance and Odor	MP (°C)	BP (°C)	Density (g/cm^3)	Solubility
Triamcinolone acetonide	(4a*S*,4b*R*,5*S*,6a*S*,6b*S*,9a*R*,10a*S*,10b*S*)-4b-fluoro-6b-glycoloyl-5-hydroxy-4a,6a,8,8-tetramethyl-4a,4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-2*H*-naphtho[2',1':4,5]indeno[1,2-*d*][1,3]dioxol-2-one	$C_{24}H_{31}FO_6$	434.50	76-25-5	White to cream-colored crystalline powder; slight odor	292-294	-	-	Sparingly soluble in dehydrated alcohol, chloroform, acetone, ethyl acetate and in methanol

Source: Budavari, S., 1996 and USP 31, 2008

Side effects

Many dermatological side-effects and carcinogenic properties have been attributed to the use of hydroquinone. In examining the FDA's arguments for banning hydroquinone, 5 categories are worthy of consideration: absorption, fertility, carcinogenicity, exogenous ochronosis, and risk-benefit [11]. Hydroquinone has been reported to cause side effects such as skin redness and burning sensations.

Retinoic acid was first used in combination with hydroquinone to enhance the penetration of hydroquinone, but was later recognized to have its own effect on the pigment. Combination therapies such as hydroquinone, retinoic acid, and corticosteroids have been used in the treatment of melasma. Over the years a combination of these substances has been altered to obtain a formulation with less severe side effects [12]. Toxicity includes a red face and a severe burning sensation, skin irritation or allergic to sunlight easily. Retinoic acid is not allowed in the EU cosmetic industry.

There are several reports on the side effects of corticosteroids. These effects of corticosteroids on bones and joints, as a result they associated with significant bone loss and increased fracture risk, effect on the gastrointestinal tract and could cause osteoporosis. Moreover, they effected on the eyes include raised intra-ocular pressure. Skin thinning and purpura have also been reported in patients [13].

Toxicity

The toxicity of hydroquinone is of interest because of its widespread occurrence in nature. It has been used in skin lightening preparation and hair dyes, and as a reducing agent in photographic developer. The acute oral toxicity of hydroquinone, retinoic acid and corticosteroids are shown in Table 3.

Table 3 Acute oral toxicity of hydroquinone, retinoic acid and corticosteroids

Compounds	Species	Oral: LD_{50} (mg/kg)	Reference
Hydroquinone	rat	302	USP MSDS. hydroquinone,
	mouse	245	Catalog No: 1324002
Retinoic acid	rat	2000	USP MSDS. retinoic acid,
	mouse	1100	Catalog No: 1674004
Betamethasone	mouse	>4500	USP MSDS. betamethasone, Catalog No: 1066009
Betamethasone-17 valerate	rat	>3000	USP MSDS. betamethasone - 17 valerate, Catalog No: 1069007
	mouse	4067	
Dexamethasone	rat	>3000	USP MSDS. dexamethasone,
	mouse	6500	Catalog No: 1176007
Hydrocortisone acetate	rat	5000	USP MSDS. hydrocortisone
	mouse	3267	acetate, Catalog No: 1317007
Prednisolone	rat	3857	USP MSDS. prednisolone,
	mouse	1680	Catalog No: 1555005
Triamcinolone acetonide	rat	6300	USP MSDS. triamcinolone
	mouse	5000	acetonide, Catalog No: 1677002

High performance liquid chromatography-mass spectrometry

The used of high performance liquid chromatography combined with mass spectrometry (HPLC-MS) or tandem mass spectrometry (HPLC-MS/MS) has proven to be the analytical of choice for most assays drug or cosmetic product. HPLC–MS and HPLC–MS/MS are used for the analysis of compounds. The first is that the HPLC mobile phase is a liquid, often containing a significant proportion of water, which is pumped through column. Typical reversed phase HPLC systems connected to MS would use some combination of water and either methanol or acetonitrile as the

mobile phase. There are limitations on the mobile phase modifiers; for example, in most cases the modifiers have to be volatile. Mobile phase modifiers are chemicals added to the mobile phase that are used primarily to improve the chromatography of the analytes of interest. Typical mobile phase modifiers would include ammonium acetate, acetic acid and formic acid. The mass spectrometer operates at a pressure of around 10^{-6} torr. The function of any interface is to remove all of the mobile phase. The second is that the majority of analytes that are likely to be separated by HPLC are relatively involatile and/or thermally labile [14]. In general, eluent flow rates of up to 1 mL/min can be used, although if an eluent with relatively high water content is employed, then a lower flow rate may be necessary. In addition, non-volatile salts and additives tend to deposit on the sampling cone resulting in a rapid decrease in instrument sensitivity.

There are various types of ionization sources that can be used as the interface between the HPLC and the mass spectrometer. The two most common sources are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). The ions are then separated by the MS system. In general, a HPLC-MS system consists of five major components, e.g., inlet, ionization interface, mass analyzer, detector and data system. A schematic diagram of a typical HPLC-MS instrument is shown in Figure 3.

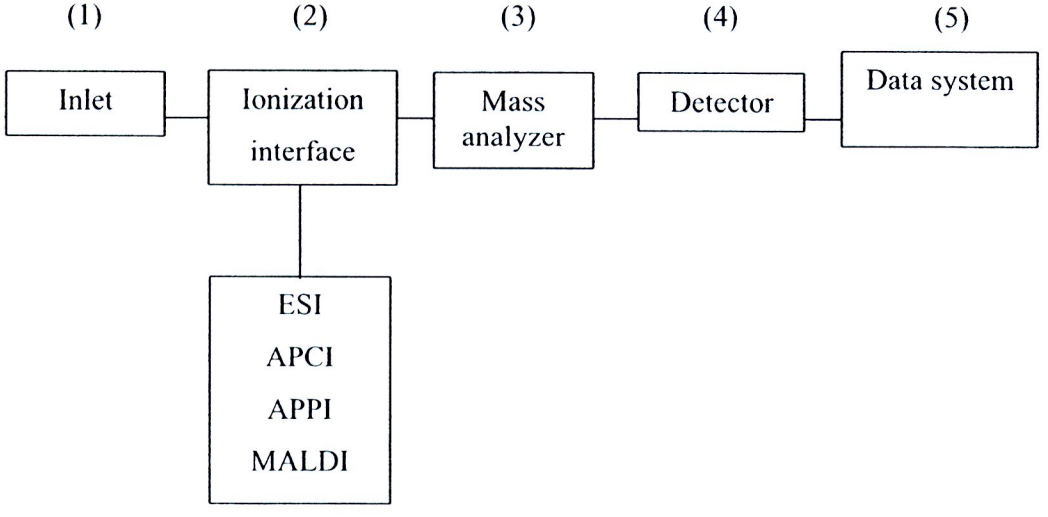


Figure 3 Block diagram of a typical HPLC-MS system

1. High performance liquid chromatography [14, 15, 16, 17]

The International Union of Pure and Applied Chemistry (IUPAC) defines chromatography as follows:

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (the stationary phase), while the other (the mobile phase) moves in a definite direction. A mobile phase is described as "a fluid which percolates through or along the stationary bed in a definite direction". It may be a liquid, a gas or a supercritical fluid, while the stationary phase may be a solid, a gel or a liquid. If a liquid, it may be distributed on a solid, which may or may not contribute to the separation process [14]. In many analyses, the compounds of interest are found as part of a complex mixture and the role of the chromatographic technique is to provide separation of the components of that mixture to allow their quantitative determination. A chromatographic system may be considered to consist of five component parts, as follows: mobile phase reservoirs, pump, injector, column and detector.

The heart of a HPLC system is the column. The column contains particles as the stationary phase. The mobile phase is pumped through the column by a pump. The mixture of compounds is injected into the flowing of mobile phase by an injector. When the mobile phase passes through the column, the molecules that adsorbs to the stationary phase migrates slowest through the column. When the mobile phase is passed through the column it enters into the detector that detects different molecules. Quantitative information is obtained from the area or height of the peak produced by the detector. A block diagram of an HPLC system is shown in Figure 4. These components are discussed in detail below.

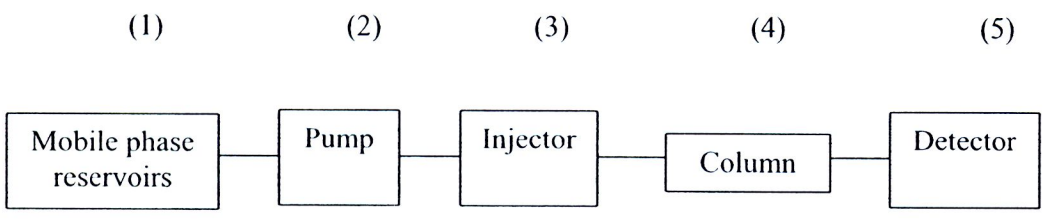


Figure 4 Block diagram of a typical HPLC system

1.1 Mobile phase reservoirs

The mobile phase supply for a liquid chromatograph usually has a capacity for at least four solvents. The solvents can be selected by a microprocessor or manually by suitable valves and these pass either directly to a dual piston pump for isocratic development or to a solvent programmer and thence to a pump for gradient elution. A mobile phase is the liquid that is pumped through the column. In reverse phase chromatography, the mobile phase consists of an aqueous buffer and a non-UV active water miscible organic solvent. Four of the most frequently used reversed phase solvents such as water, acetonitrile, methanol and tetrahydrofuran. Acetonitrile has a very low background absorbance even at wavelengths as low as 200 nm, so it is the most commonly used solvent in reversed phase separations. Typical mobile phase modifiers are undiluted acid (e.g., phosphoric, formic and trifluoroacetic) and bases (e.g., triethylamine, triethanolamine and diethylamine) as buffer system [16]. Though choice of column has the greatest effect on resolution, the mobile phase also effects selectivity and efficiency. HPLC requires a mobile phase in which the analytes are soluble.

One of the functions of HPLC-MS interface is to remove the mobile phase and a result in buffer molecules being deposited in the interface and/or the source of the mass spectrometer deposited thus consequent with a consequent reduction in detector performance. Methods involving the use of volatile buffers, such as ammonium acetate, are therefore preferred [14].

1.2 Pump

Passing mobile phase through the column at high pressure and controlling flow rate is the function of the pump. The pump must provide stable flow rates from between 10 μL/min to 2 mL/min with the HPLC–MS requirement dependent upon the interface being used and the diameter of the HPLC column. For example, the electrospray interface, when used with a microbore HPLC column, operates at the bottom end of the range, while with a conventional 4.6 mm column usually operates at the top end of the range. The most popular pump used today is the reciprocating pump. From a mass spectrometry perspective, it must deliver the mobile phase at a constant flow rate [14].

1.3 Injector

The loop injector is a convenient way of introducing a liquid sample into a flowing liquid stream and consists of a loop of a nominal volume into which sample is introduced by using a conventional syringe. When injection is required, a rotating switch is moved and the flow is diverted through the loop, thus flushing its contents onto the top of the column. Modern liquid chromatographs that are used for routine analysis usually include an automatic sampling device. The transporter carries a series of vials that alternately contain sample and washing solvent. The sampling can involve a complex sequence of operations that are controlled by a microprocessor. The syringe plunger is operated pneumatically and the syringe is first washed with solvent.

1.4 Column

Almost all HPLC columns are packed, although they can vary widely in length and diameter depending of the nature of the sample and the resolution required. They are usually manufactured of stainless steel or titanium (reputed to provide greater stability for labile materials of biological origin) and the connection to the sample valve and detector should be as short as possible and have a very small diameter to reduce extra column dispersion [15]. There are several types of phase i.e. normal phase, reversed phase, size exclusion and ion exchange. Generally, modern reversed phase HPLC columns are made by packing the column housing with spherical silica gel beads which are coated with the hydrophobic stationary phase. The stationary phase is introduced to the matrix by reacting a chlorosilane with the hydroxyl groups present on the silica gel surface. In general, the nature of stationary phase has the greatest effect on capacity factor, selectivity, efficiency and elution. The most widely used columns contain a chemically modified silica stationary phase, with the chemical modification determining the polarity of the column. A very popular stationary phase is one in which a C_{18} alkyl group is bonded to the silica surface [14, 17]. In reversed phase chromatography, the stationary phase is non-polar and the mobile phase is polar, causing polar peaks to generally elute earlier than non-polar peaks.

1.5 Detector [17]

The detector is a transducer that converts a change in sample concentration into an electrical signal. Interpretation of this record gives qualitative and quantitative data of the sample. There are several types of detector, such as UV-absorbance, Fluorescence and Refractive index detectors. The most common HPLC detectors are UV-photometers.

1.5.1 UV-absorbance detectors

These detectors measure changes in the absorbance of light in the 190-350 nm region. The same detector can be used in the 350-700 nm for absorbance in visible region. Some detectors are designed for absorbance measurements in both UV and visible region.

15.2 Fluorescence detectors

Fluorescence detectors measure change in the fluorescence of the column effluent when it is exposed to selected wavelengths of light. These detectors use filters to select the excitation and emission wavelengths.

1.5.3 Refractive index detectors

Refractive index (RI) detectors measure changes in the refractive index of the column effluent. The RI detector is a bulk property detector which responds to all compounds. The RI detector is less sensitive. RI detection is used most commonly when detection sensitive is less important such as preparative LC.

2. Mass spectrometry

Simplistically, chromatography can be regarded as the separation of the components of a mixture to allow the identification and/or quantitation of some or all of them. Identification is initially carried out on the basis of the chromatographic retention characteristic. This is not sufficient to allow unequivocal identification because of the possibility of more than one analyte having virtually identical retentions. The advantage of the mass spectrometer is that in many cases it can provide that absolute identification. It provides not only structural information from the molecule under investigation but it may also provide the molecular weight of the analyte. The mass spectrometer inlet system for liquid chromatography, often termed

the 'interface' between the two component techniques, must therefore remove as much of the unwanted mobile phase as possible while still passing the maximum amount of analyte into the mass spectrometer.

2.1 Ionization methods

Ionization methods that may be utilized in HPLC–MS include electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric photo ionization (APPI) and matric assisted laser desorption ionization (MALDI).

Electrospray ionization is a technique used in mass spectrometry to produce ions. The typical solvents for electrospray ionization are prepared by mixing water with volatile organic compounds (e.g., acetonitrile, methanol). The liquid containing the analyte is pumped through a capillary to which a high voltage (2-5 kV) is applied. Large-flow electrospray can benefit from additional nebulization by an inert gas such as nitrogen to decrease the initial droplet size. The droplets that contain an excess of positive or negative charge detach from its tip. These droplets get smaller as they approach the entrance to the mass analyzer, charged analyte molecules that can be analyzed for their mass-to-charge ratio as shown in Figure 5 [18].

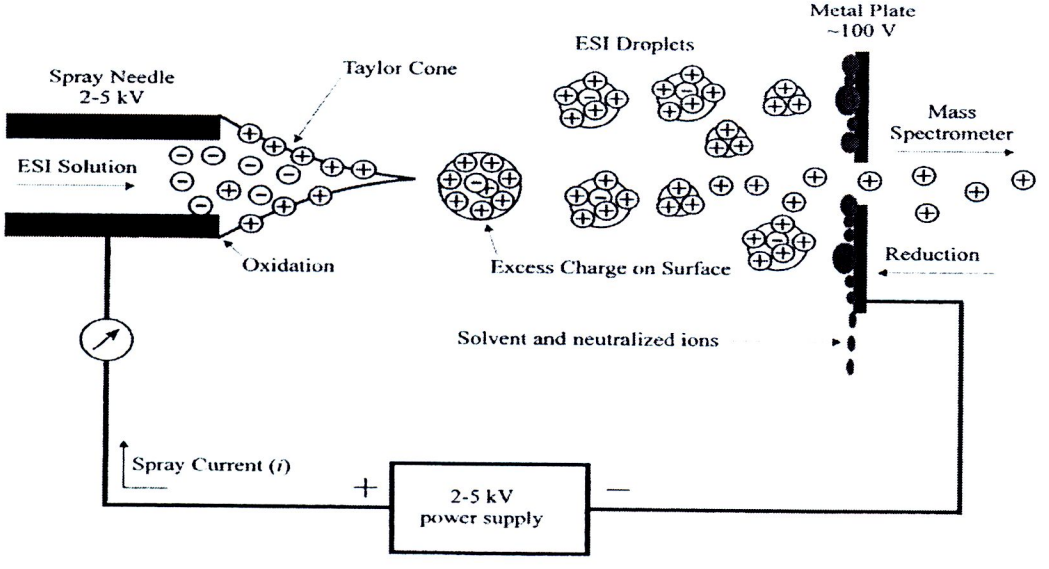


Figure 5 Schematic of the electrospray ionization process

Source: Cech, et al., 2001

In ESI-MS, the sample should be soluble in a preferably polar solvent, which can be infused, under atmospheric pressure, into the ionization source via a thin needle. If the ESI analysis is not working, or if detection limits are poor, then the analyst must determine whether the problem lies in sample preparation or separation, or whether the analyte is inherently unsuitable for analysis by ESI-MS. It may be possible to derivatize the analyte in such a way as to improve its responsiveness.

In positive ion ESI, the charge carrier can be a cation such as sodium, lithium, or ammonium, which can be added to the solution in the form of a neutral salt (such as ammonium acetate). Neutral salts are usually added to the solution to facilitate the analysis of polar, neutral analytes through adduct formation. However, positive ion ESI is most often performed with protonated solutions of acetonitrile/water or methanol/water. In this case, the charge is carried by protonated solvent clusters, which are created by reaction of the solvent with a weak acid (such as acetic, formic, or propionic acid) that is added to the solution [18]. The negative ion mode analysis also could be accomplished by using the same solvents as those used in positive ion mode, but by making the solution basic instead of acidic.

2.2 Ion separation

The mass analyzer is the heart of the mass spectrometer. The main function of the mass analyzer is to separate the ion form in the ionization source of the mass spectrometer according to their mass-to-charge ratios and subsequently records their intensities. Different physical principals can be used for separation of ions. There are numbers of mass analyzers currently available include quadrupole, time-off-flight (TOF) analyzer and quadrupole ion traps.

The quadrupole analyzer is an ideal detector for chromatography as it is capable of fast scanning and uses low voltages which make it tolerant of relatively high operating pressures, such as those encountered in HPLC–MS. The opposite pairs are connected electrically and a voltage, consisting of both radiofrequency (RF) and direct-current (DC) components, is applied, with the RF components on the two pairs of rods being 180° out-of-phase. At a specific value of these voltages, ions of a particular *m/z* follow a stable trajectory through the rods and reach the detector. A mass spectrum is therefore produced by varying the RF and DC voltages in a

systematic way to bring ions of increasing or decreasing *m/z* ratios to the detector. The quadrupole is classified as a low-resolution as shown in Figure 6 [14, 19].

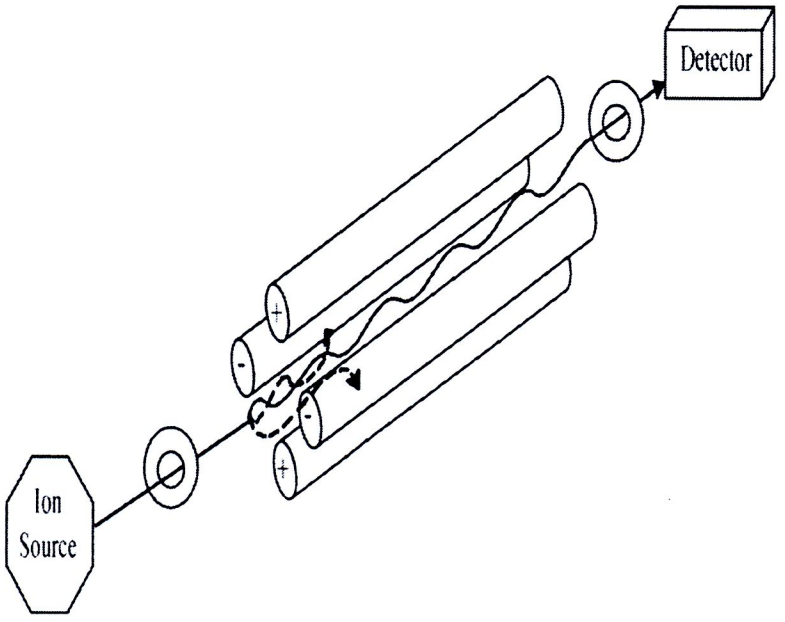


Figure 6 Representation of a quadrupole mass analyzer. Four parallel electrical rods with varying direct current and alternating radio-frequency potentials. Only one *m/z* value will possess the "right" trajectory and survive the path to reach the detector (solid line). The rest will collide with rods and will be ejected.

Source: Aneed, et al., 2009

2.3 Ion detector

The final element of the mass spectrometer is the detector. Typically, some type of electron multiplier is used, though other detectors including Faraday cups and ion to photon detectors.

Literature review

Numberous methodologies have been published on the analysis of the compounds in variety of matrices with different chromatographic techniques. Various separation techniques including gas and liquid chromatography coupled with mass spectrometry have been developed to determine glucocorticoids. Gas chromatography/mass spectrometry methods provide high sensitivity, specificity and chromatographic resolution; however, these methods require derivatization to enhance the volatility of the analytes. Many HPLC-MS methods have developed for measuring corticosteroids but there is no report on for simultaneous separation of the eight active substances using HPLC-MS.

1. Hydroquinone

Quantitative methods for the determination of hydroquinone in different matrices using a UV-VIS spectrophotometry [20], HPLC [21, 22, 23, 24] and GC-MS [25, 26, 27] have been developed.

Garcia, et al. [22] developed and validated the quantitative determination of hydroquinone in gels and creams. HPLC was carried out by reversed phase technique on a RP-18 column with a mobile phase composed of methanol and water (20:80, v/v). The linearity in the concentration range of 6.0–30.0 μg/mL presents a correlation coefficient of 0.9999, calculated by least square method. The LOD and LOQ were 0.08 and 0.26 μg/mL, respectively. Based on the preliminary spectrophotometric profile of HQ, a signal at 302.0 nm of the first derivative spectrum was found adequate for validation. The linearity between signal and concentration of HQ in the range of 10.0–26.0 μg/mL in sulfuric acid (0.1N) present a correlation coefficient of 0.9999. The LOD and LOQ were 0.14 and 0.46 μg/mL, respectively.

Chisvert, et al. [27] determined skin whitening agents such as hydroquinone in cosmetic products using GC-MS. A stock standard solution was freshly prepared in N,N-dimethylformamide (DMF). Next, 50 μL of pentachlorobenzene solution was used as internal standard, and 100 μL of BSTFA were added. Then, the vials were sealed and shaken to form derivatives. The analytes are derivatized with N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosylane. A trace TR-5MS column (length 30 m x 0.25 mm I.D., 0.25 μm film) was used. Helium was used as carrier gas at 1 mL/min. The oven

temperature program was 120 °C 1 min at 20 °C/min to 280 °C 2 min. Both injector and transfer line temperatures were 280 °C. The MS detector operated with an ionization energy of 70 eV. Extracted ion chromatogram at *m/z* 239 for hydroquinone was used.

2. Retinoic acid

There are several reports on the use of HPLC-MS [28] and HPLC-MS/MS [29] for separation of retinoic acid.

Kane, et al. [29] reported a sensitive LC-MS/MS using selected reaction monitoring to quantify retinoic acid in limited biological samples. Standard was prepared freshly on the day. The LC-MS/MS was used with a Supelco ABZ + C16 alkylamide column (100 mm x 2.1 mm, 3 µm). Mobile-phase A consisted of acetonitrile/methanol/water/formic acid (40:30:30:0.1, by vol); Mobile-phase B consisted of acetonitrile/methanol/water/formic acid (55:30:15:0.1, by vol). Then, a gradient was used. Positive-ion APCI was chosen as the most sensitive. For retinoic acid, at *m/z* 301.1 was selected in Q1 and *m/z* 205.0 in Q2. The assay quantifies over a linear range of 20 fmol to 10 pmol, and has a 10 fmol LOD.

3. Corticosteroids

One of the main drawbacks of GC in the analysis of corticosteroids is that it usually requires derivatization to improve their volatility and thermal stability. However, derivatization is not necessary in all cases [30]. Corticosteroids have been analyzed in different matrices using a GC-MS technique. This technique involves a time consuming derivatization step due to the low volatilities of the corticosteroids [31-34]. Method based on TLC [35], HPLC [36, 37], HPLC-MS [38, 39, 40, 41], and UPLC-MS/MS [42] have been developed for determination of corticosteroids. Several works on LC-MS/MS analysis of corticosteroids have been published in recent year [43, 44, 45].

A preliminary screening of cosmetic products was carried by TLC. When a sample was suspected to contain corticosteroids, the TLC analysis provided a first indication then the HPLC separation allowed the identification and its quantitation.

Gagliardi, et al. [36] was developed a chromatographic method for the analysis of 51 corticosteroids such as betamethasone 17-valerate, dexamethasone, hydrocortisone acetate and triamcinolone acetonide in cosmetic products. The HPLC

separation was carried out for identification and determination of the analytes using a Purspher RP-18 column (250 mm x 4.6 mm I.D., 5 μm), an isocratic with the mobile phase acetonitrile-water (60:40 (v/v)) or a gradient elution with a mixture acetonitrile-water in the ratio 25:75 (v/v) to 90% in 30 min, the final composition was maintained for 10 min with photodiode-array detector. For both eluents the operating condition were: flow rate, 1.0 mL/min; injection volume, 10 μL; column temperature, 25 °C; detection wavelength, 239 nm. Correlation coefficients were all greater than 0.998, the LOD ranging from 0.1-0.8 μg/mL. The recoveries were ranging from 88-98% with a RSD $\leq$ 4%.

Amendola, et al. [46] developed a screening and confirmation analytical method for the determination of glucocorticoids such as betamethasone, dexamethasone, prednisolone, triamcinolone acetonide in human urine by GC-MS (quadrupole) with EI ionization following microwave-assisted derivatization. The derivatization yields obtained by two different derivatizing mixture, namely MTSFA:NH_4I:DTE 100:2:4; and TMSim:BSA:TMCS 3:3:2 both of these mixtures under 40 min in a microwave at 900 W followed by incubation at 70 °C for 90 min. The highest yields of the derivatization process were obtained by the two-step procedure with the derivatization mixture TMSim:BSA:TMCS 3:3:2. Stock standard solutions were dissolved in methanol; stock solutions were darkly stored at -20 °C. Working standard solutions were prepared weekly and stored at 4 °C. Sample preparation was done by a C_{18} Sep-Pak for cleanup urine samples. The elute is evaporated to dryness under N_2 and then is derivatized with 50 μL of derivatization mixture (TMSim:BSA:TMCS 3:3:2) by two-step procedure. The GC-MS analysis was performed using HP5 column (length 30 m x 0.2 mm I.D., 0.11 μm film). Helium was used as carrier gas at constant flow of 0.8 mL/min and thermal program: 200 °C, 2 min, 15 °C/min; final temperature: 300 °C, 40 min, 20 °C/min; final temperature: 320 °C, 6 min. LOD of glucocorticoids in urine was 3-25 μg/L.

Andersen, et al. [45] used a solid phase extraction method for extraction of corticosteroids, i.e.,betamethasone, dexamethasone, prednisolone, triamciolone acetonide, and the triple quadrupole mass spectrometer with negative electrospray was used. A Zorbax Eclipse XDB C18 (100 mm x 2.1 mm, 1.8 μm) was used throughout the validation. Separation was performed by isocratic with a mixture of acetonitrile

and 0.1% formic acid (3:7) at flow rate 0.22 mL/min and the thermostat was set at 40°C. A 10 µL was injected. Separation of the two epimers betamethasone and dexamethasone has been addressed by using MS^3 or by separation on a Hypercarb column. Relative average recoveries from 96-103% were found. Quantification limits were demonstrated to be not higher than 1 µg/L (except 3 µg/L for prednisolone).

Luo, et al. [47] developed the simultaneous separation, identification, quantification and confirmation of betamethasone and dexamethasone in equine plasma by liquid chromatography/tandem mass spectrometry with an ESI source with the positive mode for analysis. Separation of the analytes was performed on Hypercarb column (300 mm x 2.1 mm, 5µm) using acetonitrile/water/formic acid (95:5:0.5, v/v/v) as the mobile phase. The quantifying ion was *m/z* 237 for dexamethasone and *m/z* 279 for betamethasone. The method was linear over the concentration range of 0.1-75 ng/mL. Limit of detection was 50 pg/mL and limit of quantitation was 100 pg/mL. The intra-and inter-day precisions from 0.1 to 50 ng/mL (as coefficient of variation) were less than 7%. The accuracy was in range of 97-105%. Measurement uncertainty was estimated at less than 16%.