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HPLC EVALUATION OF TYROSINE AND ITS METABOLITES

DIPLOMA THESIS

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"I declare that this thesis is my original author's work, which has been composed solely by myself (under the guidance of my consultant). All the literature and other resources from which I drew information are cited in the list of used literature and are quoted in the paper. The work has not been used to get another or the same title."

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ABSTRACT

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Title of Diploma Thesis: HPLC evaluation of tyrosine and its metabolites

Tyrosine is an important precursor of catecholamines, which are dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline). These are neurotransmitters and hormones, crucial for every living organism. Therefore, their identification and evaluation in biological material would aim to understand their function and behavior more accurately.

This Diploma Thesis is a review on how to evaluate catecholamines and their metabolites in biological matter with analytical methods used in Pharmaceutical Analysis, especially with HPLC.

First chapters present theoretical knowledge about metabolism of tyrosine and its metabolites, how a sample from an organism should be treated in order to be examined, information about HPLC, CE, GC apparatuses and the main detectors used in analysis of these compounds.

In literature review, tables are presented, concerning different sample preparation methods, HPLC details and characteristics, and finally a sum of all the analytical methods studied for this Diploma Thesis. All the articles and experimental works included in tables were chosen among many others, as they considered to provide proper results and were the most suitable for this topic. Most of these experiments were published from 1999 until 2021, so anything stated as conclusion is valid until today (2022). Pharmaceutical Analysis is a rapidly evolving field and, in the future, evaluation of metabolites of tyrosine might slightly change.

ABSTRAKT

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Název diplomové práce: HPLC hodnocení tyrosinu a jeho metabolite

Tyrosin je důležitým prekurzorem katecholaminů, tedy dopaminu, norepinefrinu (noradrenalin) a adrenalinu (adrenalin). Jsou to neurotransmitery a hormony, klíčové pro každý živý organismus. Proto je pro přesnější pochopení jejich funkce a chování důležitý jejich identifikace a hodnocení v biologickém materiálu.

Tato diplomová práce tedy představuje přehled o způsobech hodnocení katecholaminů a jejich metabolitů v biologickém materiálu metodami používanými ve farmaceutické analýze, zejména HPLC.

První kapitoly podávají teoretické poznatky o metabolismu tyrosinu a jeho metabolitů, o úpravě vzorku před analýzou a informace o přístrojích pro HPLC, CE, GC a hlavních detektorech používaných při analýze těchto sloučenin.

V literárním přehledu jsou uvedeny tabulky týkající se různých metod přípravy vzorků, podrobnosti o HPLC podmínkách a nakonec souhrn všech analytických metod studovaných v této diplomové práci. Do tabulek byly zahrnuty články vybrané z mnoha dalších, poskytující nejlepší výsledky, a tedy považované za nejvhodnější. Většina těchto experimentů byla publikována od roku 1999 do roku 2021, takže vše, co je uvedeno v závěru, platí až do současnosti (2022). Farmaceutická analýza je rychle se vyvíjející obor a v budoucnu by se přístup k hodnocení metabolitů tyrosinu mohl mírně změnit.

List of Abbreviations

3-MT	3-methoxytyramine
5-HIAA	5-hydroxyindoleacetic acid
ACN	Acetonitrile
AD	Aldehyde dehydrogenase
APCI	Atmospheric-pressure chemical-ionization interface
AR	Aldehyde reductase
ASTED	Automated sequential trace enrichment of dialysates
BGE	Background electrolyte
B-h DIPs	Hollow dummy template imprinted boronate-modified polymer
BSTFA	N,O-Bis(trimethylsilyltrifluoroacetamide)
CA	Catecholamines
CE	Capillary electrophoresis
COMT	Catechol-O-methyltransferase
CSF	Cerebrospinal fluid
DA	Dopamine
DAD	Diode array detection
DEEMM	Dibenzylethoxymethylene malonate
Depr.	Deproteinization
DHBA	3,4-dihydroxybenzylamine
DHMA	3,4-dihydroxymandelic acid
DHPA	3,4-dihydroxyphenylpropanoic acid
DHPG	3,4-dihydroxyphenylglycol
DOPAC	3,4-dihydroxyphenylacetic acid
DOPAL	Dihydroxyphenylacetaldehyde
DOPEG	Dihydroxyphenylethylene glycol
DOPEGAL	Dihydroxyphenylglycolaldehyde
DOPET	3,4-dihydroxyphenylethanol
DT	Diploma thesis
E	Epinephrine
ED	Electrochemical detection
EDTA	Ethylenediaminetetraacetic acid
ES	External standard
ESI	Electrospray interface
FASI-CE	Field-amplified sample injection CE
FD	Fluorescence detection
Fe_3O_4@APBA	Aminophenylboronic acid functionalized magnetic nanoparticles
FITC	Fluoresceine-5-isothiocyanate
GC	Gas chromatography
HILIC	Hydrophilic interaction liquid chromatography
HMBA	4-hydroxy-3-methoxybenzylamine
HMDS	Hexamethyldisilazane
HPC	Hydroxypropylcellulose
HPLC	High-performance liquid chromatography
IEC	Ion-exchange chromatography
IS	Internal standard
L-DOPA	L-3,4-dihydroxyphenylalanine
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantitation

LRSC	Lissamine rhodamine B sulfonylchloride
MA-co-EGDMA	Methacrylic acid-co-ethylene glycol dimethacrylate
MAO	Monoamine oxidase
MBHFBA	*N*-Methyl-bis(heptafluorobutyramide)
MBTFA	*N*-Methyl-bis(trifluoroacetamide)
MES	2-(*N*-morpholino)ethanesulfonic acid
MHPG	3-methoxy-4-hydroxyphenylglycol
min.	Minute(s)
MIP-SPE	Molecular imprinted polymer SPE
M-PST	Monoamine-sulfating phenolsulfotransferase
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSTFA	*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide
MT	Migration time
MTBSTFA	*N*-(tert-butyldimethylsilyl)-*N*-methyltrifluoroacetamine
NDA	Naphthalene-2,3-dicarboxaldehyde
NE	Norepinephrine
NR	Not referred
OSA	1-octanesulphonic acid sodium salt monohydrate
PBS	Phosphate-buffered saline
PCE-PS	Polymeric crown ethers with polystyrene
PDDAC	Poly(diallyldimethylammonium chloride)
PFHA	Perfluoroheptanoic acid
PFPA	Pentafluoropropionic anhydride
PFP-Br	Pentafluorobenzyl bromide
PFSPE	Packed fiber solid-phase extraction
PP	Protein precipitation
QuEChERS	Quick, easy, cheap, effective, rugged, and safe
Ref.	Reference
SDS	Sodium dodecyl sulfate
SIM	Selected ion monitoring
S/N	Signal-to-noise
SOS	Sodium octyl sulfate
SPE	Solid-phase extraction
TEA	Triethylamine
TEC	Trace enrichment cartridge
TFAA	Trifluoroacetic anhydride
TMCS	Trimethylchlorosilane
TMSI	*N*-trimethylsilyl-imidazole
TOABr	Tetraoctylammonium bromide
TOF	Time-of-flight
TPA	Tripropylamine
Tyr	Tyrosine
UPLC	Ultra performance liquid chromatography
UV	Ultra-violet
VMA	Vanillylmandelic acid
μBAMC	Miniaturized boronate affinity monolithic column

TABLE OF CONTENTS

1. INTRODUCTION

The current Diploma Thesis (DT) is a literature review of possible analytical methods for determination of tyrosine metabolites, catecholamines specifically, in biological material.

Tyrosine is a non-essential amino acid, able to be produced naturally in the body from the precursor phenylalanine. It may also be obtained from the diet, and in this case, metabolism of phenylalanine is not required much. Tyrosine is an important precursor to certain neurotransmitters, called catecholamines, which are norepinephrine (NE), epinephrine (E), and dopamine (DA), to thyroid hormones and to melanin[1]. In this paper, compounds of interest are the catecholamines. These are naturally occurring neurotransmitters in an organism, responsible for vital functions. They may also act as biomarkers for different diseases, along with their metabolites[2]. Thus, their identification, evaluation, and quantification can contribute to a better understanding of living organisms and to the diagnosis of many disorders.

The main analytical method included in this work which is capable to reach this aim is High-Performance Liquid Chromatography (HPLC), followed by Capillary Electrophoresis (CE) and Gas Chromatography (GC). Each of the above instruments has its own principle, particularities, and specifications. The conditions that should prevail during their instrumentation and handling are crucial to be comprehended for the optimum operation and to obtain adequate results.

Also, in this DT are included the methods that are applied to treat and prepare the biological material in such a manner, as to extract the desired metabolites and decrease the interferences with other occurring compounds within the sample, for the upcoming analysis. Among them, Solid-Phase Extraction (SPE), Liquid-Liquid Extraction (LLE), and Protein Precipitation (PP) are the most important and utilized thoroughly.

2. AIM OF WORK

Evaluation of tyrosine's metabolites is a topic of major importance, which concerns scientists over the years, since the 50s. There are plenty of experiments conducted and literature is enormous. Therefore, the main objects of this Diploma Thesis are:

- to create an accurate and illustrative review of them,
- to present the basic sample preparation methods,
- to analyze the most proper HPLC conditions,
- to summarize features of different analytical methods, along with the principal detectors used, in order to obtain the desired results, through different scopes (highest sensitivity, most rapid method, etc.).

3. THEORETICAL PART

3.1. CATECHOLAMINES

3.1.1. CATECHOLAMINE METABOLISM

Catecholamines are neurotransmitters, which are derived from L-tyrosine. The 3 main catecholamines are dopamine (DA), norepinephrine (NE) and epinephrine (E). They are synthesized in the brain, adrenal medulla, and neurons of the sympathetic system. They are stored in chromaffin cells of the medulla and in storage vesicles at nerve terminals[3]. As stated by their name, they contain a catechol group, along with an amine. Epinephrine has a secondary amine, while norepinephrine and dopamine have a primary amine. As previously mentioned, L-tyrosine is the main precursor, which is converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase. Then, dopa decarboxylase removes carboxylic group from L-DOPA to obtain dopamine. Dopamine β-decarboxylase and phenylethanolamine-*N*-methyltransferase convert dopamine to norepinephrine and norepinephrine to epinephrine, respectively. Conversion of NE to E mainly happens in the adrenal medulla[4]. There are 2 main enzymes that lead to breakdown of the 3 catecholamines, monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT). The first is responsible for deamination of DA to dihydroxyphenylacetaldehyde (DOPAL) and of NE and E to dihydroxyphenylglycolaldehyde (DOPEGAL). Both DOPAL and DOPEGAL are intermediates with short half-life and are metabolized by aldehyde dehydrogenase (AD) and aldehyde reductase (AR). Concerning DA's metabolites, as DA does not contain β-hydroxyl moiety, it is converted more often to 3,4-dihydroxyphenylacetic acid (DOPAC) by AD (or aldose reductase), rather to 3,4-dihydroxyphenylethanol (DOPET) by AR. In contrast, NE's and E's metabolite is converted to 3,4-dihydroxyphenylglycol (DHPG) by AR and to 3,4-dihydroxymandelic acid (DHMA) by AD, where the first is preferred, as NE and E contain a β-hydroxyl group[2]. Then, there is the other important enzyme for the metabolism of CA, COMT. This is responsible for the conversion of DHPG to 3-methoxy-4-hydroxyphenylglycol (MHPG), the main metabolite of NE in human plasma, which is oxidized to the final product of this metabolism, vanillylmandelic acid (VMA) into the liver and excreted in the urine. Another function of COMT is the metabolism of DA, NE, and E to 3-methoxytyramine (3-MT),

normetanephrine (NMN) and metanephrine (MN), respectively. DOPAC is converted to HVA, the end-product of DA metabolism through COMT. HVA can be obtained by oxidative deamination of 3-MT as well. Besides conversions to deaminated and *O*-methylated metabolites, there is also glucuronidation and sulfation that both catecholamines and their metabolites undergo. The enzyme responsible is monoamine-sulfating phenolsulfotransferase (M-PST), which is expressed mostly in the small intestine[5] (Fig. 1).
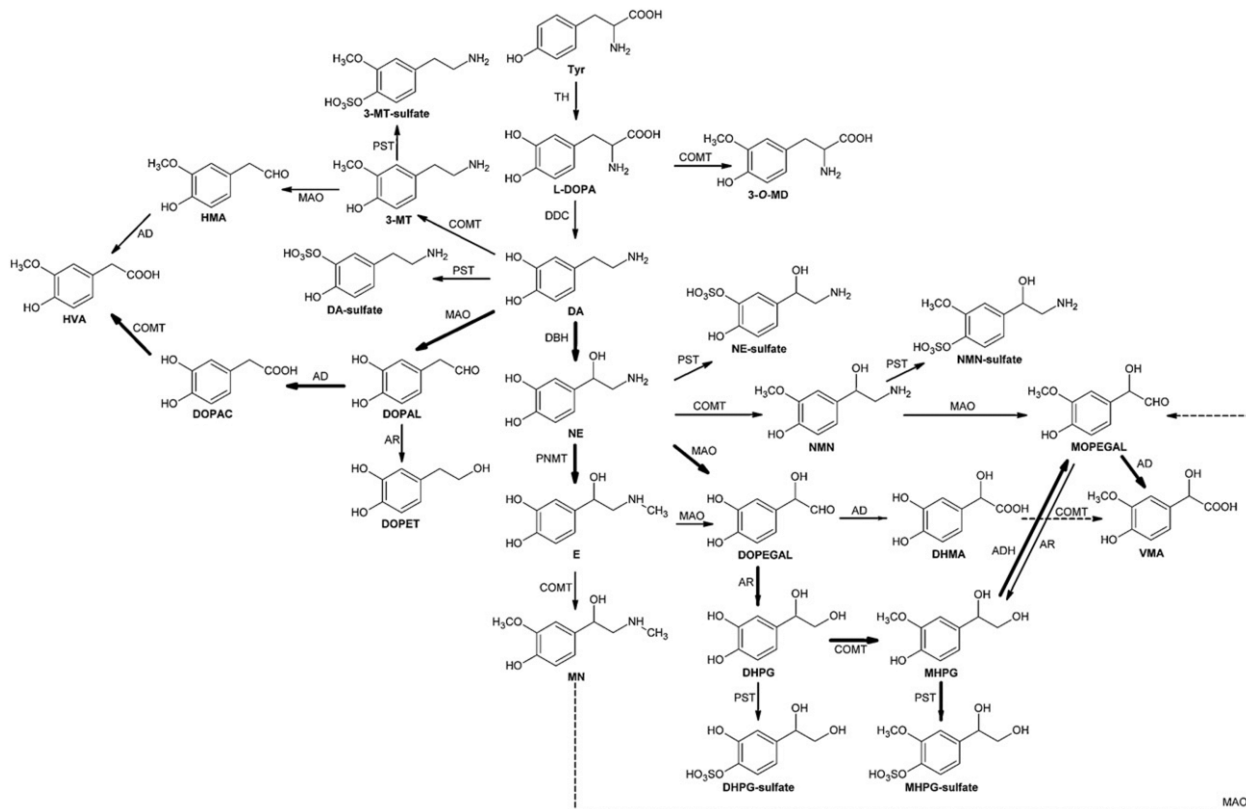
Figure 1: Metabolism of tyrosine.

3.1.2. CHEMICAL PROPERTIES

Catecholamines contain a catechol and an amine group, making them amphoteric and highly hydrophilic compounds. In acidic conditions, catechol moiety is stable, while amine moiety is protonated. In neutral and basic environment, catechol is oxidized to quinone, therefore they are electroactive as well. Since they can be both acidic and basic, their separation becomes demanding[2].

Some chemical and physical properties are stated below (Table 1):

Table 1: Chemical and physical properties of catecholamines.

	Dopamine	Norepinephrine	Epinephrine
Molecular formula	$C_8H_{11}NO_2$	$C_8H_{11}NO_3$	$C_9H_{13}NO_3$
Molecular Weight	153.18	169.18	183.204
Melting point (°C)	128	217	211.5
pKa	8.93	8.58	8.59

- Solubility[6]:

Dopamine hydrochloride (the salt that is used in practice) is freely soluble in water, soluble in alcohol, sparingly soluble in acetone and in methylene chloride.

Norepinephrine hydrochloride (the salt that is used in practice) is very soluble in water, slightly soluble in alcohol. Norepinephrine tartrate is freely soluble in water, slightly soluble in alcohol.

Epinephrine tartrate (the salt that is used in practice) is freely soluble in water, slightly soluble in ethanol (96 per cent).

- Effect on organism:

Dopamine has positive inotropic activity, acts as an agonist to α1 receptors of vascular smooth muscles, causing vasoconstriction and increasing vascular resistance. It also stimulates β1 receptors in myocardium, increasing heart rate and so, heart output. Finally, acts as an agonist to dopaminergic receptors that can be found in different areas in the body (kidney and brain among them), leading to an increase of renal blood flow, urine excretion, and is considered the chemical responsible for the feeling of reward. Its deficiency is associated with Alzheimer's disease. Also, dopamine regulates the extrapyramidal system and inhibits unnecessary movements[7]. Finally, inhibits prolactin release and stimulates release of growth hormone[8].

Norepinephrine has effect on α receptors in iris, arteries, veins, sphincter, and urinary bladder and leads to vasoconstriction. Agonistic activity in β1 receptors leads to positive inotropic, chronotropic, and dromotropic effects, while activity on β2 receptors triggers smooth muscle dilatation of bronchioles and vessels[9]. Also, it makes alertness on the brain and its deficiency is associated with depression and anxiety[10].

Epinephrine is related to the fight or flight response, which makes an individual alert. It acts on vascular and α receptors, causing vasoconstriction, thus, increased blood pressure. It has a similar effect on conjunctiva α receptors, leading to less secretion of aqueous humor and decreased intraocular pressure. Lastly, it has agonistic activity in β1 receptors, which is responsible for increased myocardial contractility and bronchodilation. Furthermore, it raises blood glucose levels and breaks down fats[10]. As a drug, it can be used to treat allergic reactions, hypersensitivity, respiratory distress caused by bronchospasm, and in cases of cardiopulmonary resuscitation, to avoid cardiac arrest. It is given parenterally[11].

3.2. BIOLOGICAL SAMPLE PREPARATION

When working with biological fluids, it is important to follow specific steps, in order to get sufficient, reliable, and reproducible results. Among them are sampling, sample preparation, separation, detection along with identification, and finally calibration followed by quantification[12]. Eventually, all currently used methods perform validation.

Initially, a sample must be collected from organisms (sampling). The 2 main types of liquid used for analysis are blood and urine. Regarding blood, it is more common to isolate either plasma or serum from whole blood and utilize them as samples, as they are simpler and do not need as much sample preparation as whole blood. Generally, after separation, it is better to analyze them instantly, otherwise can be stored in a freezer at around -21ºC. Urine should also be immediately utilized after collection or may be stored in a freezer as well. To a less extent, the sample is taken from cerebrospinal fluid (CSF) which is found in the spinal cord, or from any kind of tissue (biopsy), mostly brain tissue concerning catecholamines. CSF and biopsies can be stored in a freezer[12].

Pure biological material is difficult to handle during analysis with the previously mentioned methods. They contain many other substances besides target analytes and the concentration of the latter is usually very low. Thus, the step of sample preparation has a determinant role for analyte detection during biological sample analysis. The main objective of sample preparation is to remove proteins that may clog the chromatographic system and lead to increased back-pressure and possible destruction of the apparatus, as well as other possibly existing substances of the matrix that may interfere with the procedure. Another goal is to increase the concentration of an analyte

when it cannot be detected during quantitative analysis and to exchange the aqueous biological liquid with a more convenient solvent for the system, prior to injection of the sample[12].

There are 3 main types of sample preparation: Protein Precipitation (PP), Liquid-Liquid Extraction (LLE), and Solid-Phase Extraction (SPE).

3.2.1. PROTEIN PRECIPITATION

Protein Precipitation (Fig. 2) is a method that can be used in order to remove proteins from the biological fluid. There are also other techniques with the same objective, like ultrafiltration and enzymatic method, which are less common. PP is applied more in serum and plasma samples, which contain large amounts of proteins, in contrast with urine, that is almost free of proteins. The procedure takes place in a centrifuge tube, where the samples are placed with a volume in the range of 0.1 to 1.0 ml. Inside this tube, there is also a water-miscible solvent, like acetonitrile which is the most efficient and widely used. Such solvents, which are called precipitants, are miscible with biological fluids, create a single-phase system, while proteins become less soluble with them, thus, they precipitate. Next, the tube is shaken for some time and then centrifugation happens. At the end of the procedure, supernatant and precipitated proteins can clearly be observed and the supernatant is taken for analysis[12].
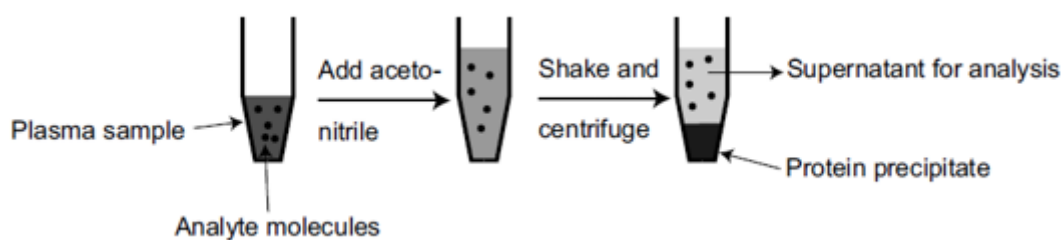
Figure 2: Protein Precipitation.

3.2.2. LIQUID-LIQUID EXTRACTION

A more traditional sample preparation procedure is Liquid-Liquid Extraction (Fig. 3). It is based on the extraction of analytes from the aqueous solution, which is the biological fluid, to an organic solvent. It takes place in a centrifuge tube, where serum, plasma, or urine is positioned, along with an organic solvent, called extraction solvent, which should be immiscible with the natural material, for a two-phase system to be formed. First, the tube is shaken for 1 to 10 minutes and is let to stand to obtain the two phases.

During shaking, small droplets of the organic solvent are created, which catch the analytes from the aqueous phase. Then, the tube is centrifuged, particularly when working with biological material, where separation of phases is difficult to achieve. Finally, the extract (the organic phase containing the analytes) is collected, the solvent is evaporated by a stream of nitrogen, the residue is redissolved in a suitable solvent, and injected into the system.

This method is governed by the partition ratio. The partition ratio is equal with the concentration of the analyte extracted into the organic phase to the concentration of the analyte in the aqueous phase; $K_D = \frac{[A_{Organic}]}{[A_{Aqueous}]}$. The higher this value is, the better is the extraction[12]. This ratio is mainly suitable for neutral molecules. For acidic and basic compounds though, important parameter is the pH of the aqueous sample, as only uncharged portion may be extracted into the organic phase. Thus, the concept of distribution ratio is applied; $D = K_D \cdot \frac{[H^+]}{[H^+] + K_a}$ for acids and $D = K_D \cdot \frac{K_a}{[H^+] + K_a}$ for bases, where K_α is the dissociation constant. It is discernible that for acids, pH should be low, and for bases, pH should be high to avoid ionization and maintain D as close to K_D as possible[12].
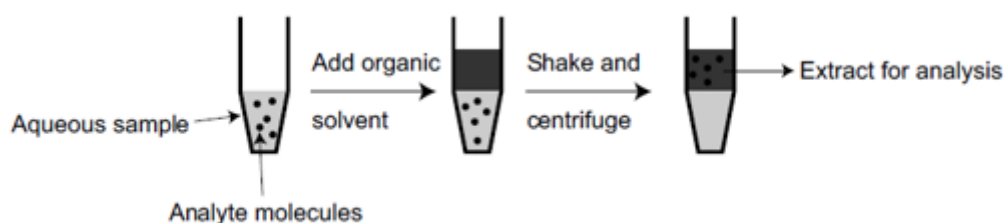
Figure 3: Liquid-Liquid Extraction.

3.2.3. SOLID-PHASE EXTRACTION

The final method is Solid-Phase Extraction (SPE) (Fig. 5). Material used (Fig. 4) is a small polypropylene column that contains a stationary phase, which is fixed at the end of the column by two polyethylene filters sideways to it. The stationary phase consists of particles of 40 to 50 µm, making possible the passage of biological material that might be highly viscous and significantly reducing back-pressure. These particles are either made from silica or organic polymers. Silica particles are highly porous, making the diffusion of low molecular weight substances through them easy, in comparison with high molecular weight compounds which almost do not pass through the pores and

can be flushed from the column. Apart from the SPE column, there is a vacuum manifold at the end part of the column, capable of applying vacuum to direct the flow of liquids. There is also a valve between the column and the vacuum manifold, which turns on and off the vacuum, according to the stage of the procedure. Finally, vials are attached to the end of columns, for multiple extractions of samples.
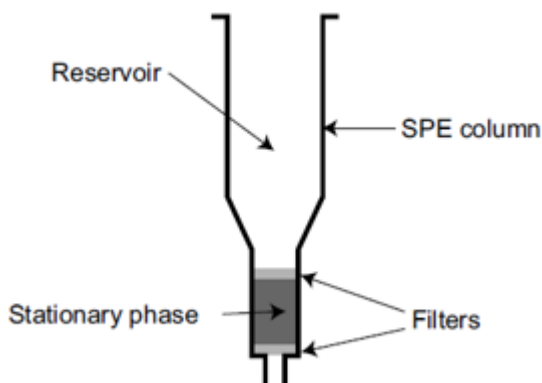
Figure 4: Structure of a Solid-Phase Extraction material.

For the appropriate application of an SPE, there are 4 actions that must be followed each time in order. First, it should be mentioned that at the "rest" state (dry state), the functional moieties of the column are disabled and must be activated. A polar organic solvent, mostly methanol is utilized to achieve this. Then, methanol is flushed with aqueous buffers or water, as it can significantly influence the elution of analytes later since it is a strong eluent. This process is called conditioning. The next step is sample loading, where biological material is deposited in the column and analytes create different kinds of bonds with the stationary phase. As biological liquids contain different types of compounds that may affect analysis, washing is needed to remove them, by keeping the analytes in the column. Finally, in order to extract the analytes from the column, an eluent is used, which can destroy the interactions between these substances with the stationary phase.

There are 4 main types of SPE: Reversed-Phase, Ion-Exchange, Mixed-mode and normal-SPE.

In Reversed-phase SPE, analytes that are extracted are non-polar. The main interaction in this type is hydrophobic one between the stationary phase and the analytes, and a minor bond between the free silanol moieties and some analytes is presented as well.

The stationary phase can be made from silica (C_{18} or C_8 mainly) or from polymers (typically divinylbenzene and *N*-vinylpyrrolidone as monomers).

Then, there is Ion-Exchange SPE. Interactions that govern this principle are ionic and it is used mostly for the elution of acidic and basic analytes. Here, stationary phases are either strong or weak, cation or anion exchangers. Strong exchangers have a charge in the whole range of pH, in comparison with weak exchangers, which have a charge in specific pH regions, so, they can be turned "on" and "off". For elution to take place, the stationary phase and analytes must be oppositely charged.

Mixed-mode SPE is the next type, which uses both hydrophobic (main) and ionic interactions. The silica-stationary phase contains octyl groups, together with an exchanger, for hydrophobic and ionic interactions, respectively.

Finally, there is normal-phase SPE, which is not used for the analysis of catecholamines, and it will not be further explained[12].
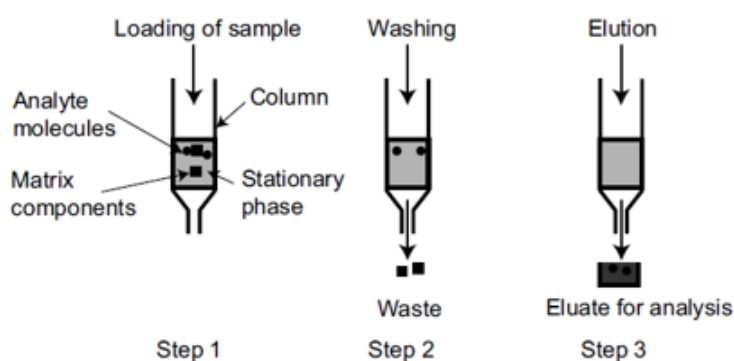
Figure 5: Solid-Phase Extraction.

What follows sample preparation is separation, where HPLC, CE, and GC procedures can be chosen and detection, where detectors like MS, UV, electrochemical, and fluorescence apparatuses are able to identify the molecules, each working with different principles[12]. Details about these two steps can be found in next chapters.

3.2.4. CALIBRATION AND QUANTIFICATION

Finally, for completion of the analysis, calibration and quantification should take effect[12]. Initially, one should understand the term calibration, which indicates the procedures that happen so to establish the relation between values found by the analytical apparatus with known values of analyte[13]. There are two types, external (ES)

and internal (IS) standards, with the latter being more used in bioanalysis. Internal standards provide more precise results than external standards and are employed for the extraction of the analyte. Their structure, chemical behavior, and molecular weight should be similar to the analyte's to provide similar signals and outcomes. Then, for quantification, standard solutions are made to get a calibration curve and a regression line equation. A standard solution is a solution from a drug-free biological matrix, where a portion of the stock solution is added. Stock solutions are fixed solutions with known concentrations of the desired analyte. This concentration should be similar with the expected one from the real sample. In case of calibration, calibration range should cover the expected concentrations. The same amount of IS is added to standard solutions and the real sample, which are treated, handled, and stored in the same manner. Before quantification, it should be checked that sample preparation removes possible chemicals that could affect analysis, by using a drug-free real sample. Then, the response ratio is calculated (peak area/height of analyte to peak area/height of IS) and the calibration curve (response ratio to concentration) is constructed. From this, the concentration of analyte in a real sample can be estimated[12].

3.3. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

High Performance Liquid Chromatography is the most used separation method for compounds. It is based on separation on a solid stationary phase, using a liquid mobile phase. Mobile phase is crucial for the overall performance of chromatography and the resolution of analytes. There are different mechanisms of separation methods, according to the type of stationary phase used, which are partition, adsorption, ion exchange, and size exclusion. According to these mechanisms, normal-phase chromatography, reversed-phase chromatography, ion-exchange chromatography, size-exclusion chromatography, and affinity chromatography can be distinguished.

3.3.1. NORMAL-PHASE CHROMATOGRAPHY

Normal-phase chromatography consists of a polar stationary phase and a non-polar mobile phase. It is mostly used to separate highly polar compounds, as they have greater affinity to the stationary phase than the mobile one. Commonly used stationary phases are silica, porous graphite, and alumina, which work based on the mechanisms of

adsorption and partition. This was the first type of HPLC but is not so common nowdays[12].

3.3.2. REVERSED-PHASE CHROMATOGRAPHY

Reversed-phase chromatography uses a hydrophobic stationary phase, and a more polar mobile phase. The main separation mechanism is partition of the analytes between the stationary and the mobile phase. It is reasonable that the more hydrophobic an analyte is, the longer is its retention time. Columns may contain many kinds of non-polar stationary phases, like modified silica (mostly C18), polystyrene-divinylbenzene, activated carbon, and diamond. There are two main bonds that make this principle possible: hydrophobic bonds and bonds between more polar groups, especially amines with silanol groups-important interaction for catecholamines[12].

3.3.3. ION-EXCHANGE CHROMATOGRAPHY

Another HPLC separation method is ion exchange chromatography. This is based on charges of the components. Stationary phase contains ionic species, to which counter-ions are attracted and make ionic bonds. There are 2 types of stationary phases, in IEC called ion exchangers: the cation and the anion exchanger, similarly as in ion-exchange SPE. Retention time depends mainly on the analyte's size and charge. In case of a small compound with a high charge, the retention increases. In order to elute the analyte, pH and/or ionic strength may be altered. To make this possible, someone can add organic solvent, increase the concentration of buffer in the mobile phase or use another buffer with a higher affinity to the stationary phase than the analyte[12].

3.3.4. SIZE-EXCLUSION CHROMATOGRAPHY

One more type is size-exclusion chromatography. The principle is grounded on the size of molecules. It differs from the previous methods, as the stationary phase is not different in terms of polarity from the mobile phase. The stationary phase is a porous solid support, fixed in the column, usually porous silica or polymer, while the mobile phase is liquid. Analytes should dissolve in the mobile phase but should not interact with the matrix. Fact is that small molecules have higher retention time than larger molecules. Pore size must correspond to a suitable molecular weight range for correct action. Tiny molecules below this range are not separated and large molecules above the range do not manage to penetrate, so, separation does not take place[12].

3.3.5. AFFINITY CHROMATOGRAPHY

Finally, there is affinity chromatography, which is applied to separate large biomolecules. The stationary phase contains an immobilized molecule, the affinity ligand, able to create selective interactions with the compounds of interest. These represent natural interactions, that may be detected in organisms. Ligands may be antibodies, substrates, lectins, and DNA, among others, to retain antigens, enzymes, sugars, and complementary DNA sequences, respectively. The sample is dissolved in the mobile phase (called application buffer) of appropriate pH, composition, and ionic strength, which is injected into the column. Interactions between ligands and desired biomolecules arise. Then, the column is washed to eliminate unfavorite substances, followed by elution with an elution buffer, for the recovery of the retained biomolecules[14].

3.3.6. HPLC APPARATUS

A typical HPLC apparatus (Fig. 6) consists of a reservoir, which is the source of the mobile phase, a pump, an injector, a column, a detector, and finally a data collection device, which is mainly a computer including a suitable chromatography software[12].

The main function of a pump is to supply the system with the mobile phase from the reservoir, with a constant flow rate. There are different kinds of pumps, with the piston pump being the most used. Generally, there are 2 types of pumping systems. First, there is isocratic elution, which occurs when the pump provides mobile phase to the column with the same composition throughout the whole run. When the composition of the mobile phase changes during the run, there is gradient elution. This technique is used when high differences in the retention time of compounds in the mixture are expected. No overlapping peaks from fast eluting compounds occur in the chromatogram, as well as no broad peaks from the more retarded eluting compounds, in contrast with isocratic elution. Gradient elution runs by starting the elution with the weaker eluting composition of the mobile phase, for fast eluting compounds to separate satisfactorily and then increasing the strength of the mobile phase, for quicker elution of later eluting substances[12].

A column is the main and most important part of an HPLC apparatus. It is a steel tube of a specific length, containing particles of specific size. For high efficiency, the main

concept is the need for many theoretical plates (N), which is an indicator of efficiency. N is directly related to the size of particles in the column; the smaller the size, the more the theoretical plates are. Also, to avoid high pressure within the HPLC system (back-pressure), a short length of the column is required, when small particles exist. This combination leads also to less consumption of mobile phase and shorter analysis time. Another factor that increases the reliability of an adequate separation is the narrow particle size distribution of column material. Concerning the above, a typical column used in HPLC analysis has a length of 15-25 cm consisting of 5 µm particles[12].
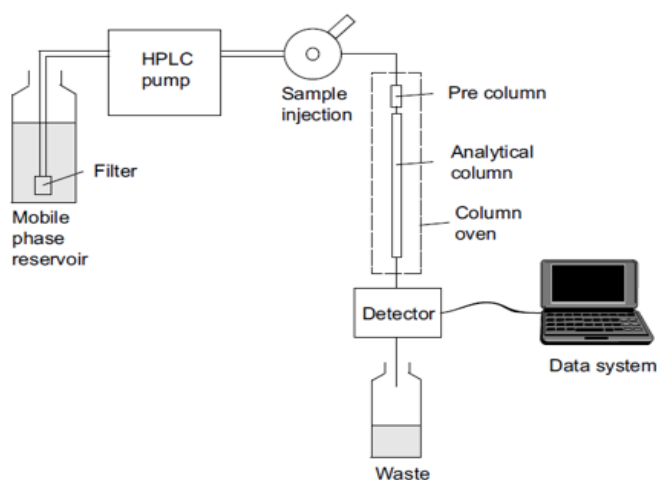
Figure 6: HPLC apparatus.

3.3.7. HPLC FACTORS

Retention time (t_R) (Fig. 7) is the time from the injection of the sample to the top of the peak in the chromatogram. The volume of the mobile phase needed to elute this compound is called retention volume (V_R). Retention times generally depend on the flow rate of mobile phases and the column length[12].

Another factor is **distribution constant** (K_c), where $K_C = C_s/C_m$. C_s is the concentration of analyte in the stationary phase and C_m is the concentration of it in the mobile phase. The higher the K_c, the higher the affinity to the stationary phase[12].

A practical expression is the **retention factor** (k), where $k = K_c \frac{V_s}{V_m}$. It expresses the amount of analyte in the stationary phase to its amount in the mobile phase[12]. The retention factor can be calculated using the chromatogram directly through the equation:

23

$k=\frac{t_R-t_M}{t_M}$, where the numerator expresses how long the compound remains in the stationary phase, and t_M states the elution time of unretained solutes[12].

Column efficiency (Fig. 8) is characterized by the theoretical plate number (N), which expresses the peak broadening in the column. In European Pharmacopeia for practical reasons, N is calculated as $N = 5.54\ (t_R/W_h)^2$, where W_h is the width of a peak in the middle of its height. N is greater when peaks are narrower regarding the retention time and thus, column efficiency is high. N is also proportional to the length of the column; as length increases, N increases too, as long the other factors are constant[12].

Separation factor α represents the **selectivity** of a system and is expressed as $\alpha = k_2/k_1$, where k_2 represents the retention factor of the compound eluted later and k_1 is the retention factor of the first eluted compound. The higher α is, the more convenient is the separation[12].

Peak symmetry (Fig. 9) is an essential characteristic of a chromatogram, as Gaussian curves are needed for sufficient separation. One can calculate peak symmetry with the next equation: $A_s = w_{0.05}/2d$, where $w_{0.05}$ is the width of the peak at 1/20 of its height and d is the distance created between the perpendicular from the top of the peak ending to this width, to the leading edge of the width. When A_s is equal to 1, there is peak symmetry. If $A_s<1$, then there is peak fronting, and when $A_s>1$, there is peak tailing[12].

Resolution (Fig. 10) is the grade of separation between two adjacent peaks. In European Pharmacopeia, it is described by the equation: $R_s=1.18\frac{t_{R_2}-t_{R_1}}{w_{h_1}+w_{h_2}}$, where t_{R1} and t_{R2} are the retention times of peak no.1 and no.2 respectively and w_{h1} and w_{h2} are the widths at the middle of peak heights no.1 and no.2, in that order. Separation at R = 1.0 is not sufficient (around 94%), while baseline separation exists at R = 1.5 and more[12].

An "overall" equation that relates resolution with retention factor, separation factor, and plate number is the following:

$$R_s=\frac{1}{4}\sqrt{N}\,\frac{(a-1)}{a}\,\frac{k}{(k+1)}$$

From the above equation, some significant conclusions are made:

- For satisfactory interaction of the analyte with the stationary phase, k should be between 3-10. The higher the retention factor is, the higher the resolution.

- The plate number should be increased by 4 times its value, in order to double the resolution, as the resolution is directly proportional to the square root of the plate number.

- As α increases, resolution increases[12].
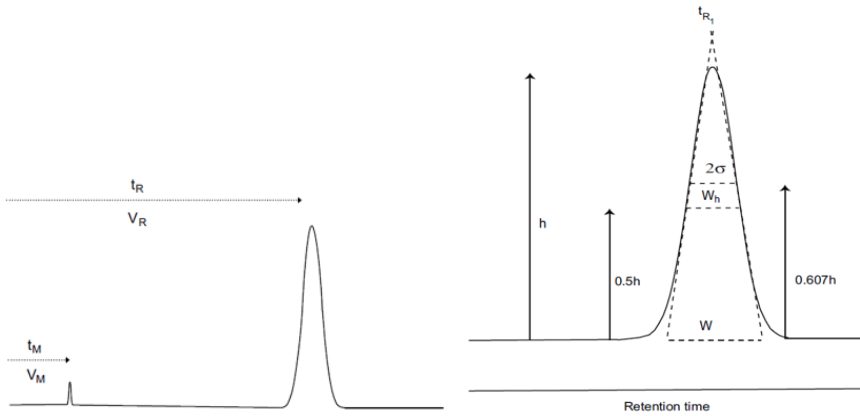
Figure 7: retention time.

Figure 8: column efficiency.
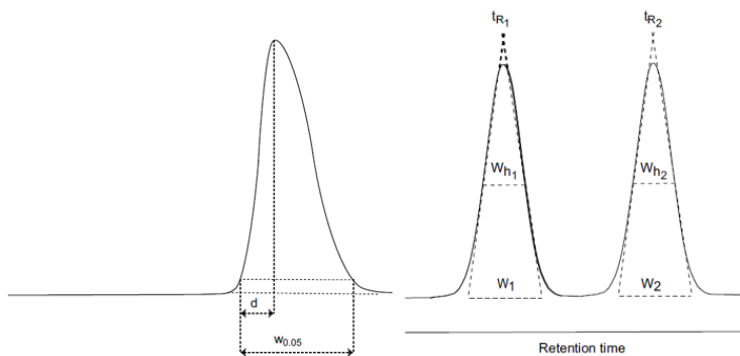
Figure 9: peak symmetry.

Figure 10: resolution.

3.4. CAPILLARY ELECTROPHORESIS

Capillary Electrophoresis (Fig. 11) is a separation method that works under the principle of Electrophoresis, meaning the movement of particles within a liquid under an applied electric field. The main apparatus of a typical Capillary Electrophoresis system consists of a capillary, the buffer, a high-voltage power supply, a detector, a sample introduction system, an output device, and sometimes a temperature control device[15]. The capillary column is a fused-silica capillary that can be either coated or uncoated[15]. The buffer, which has a specific pH, is an important part of the system, as it carries the solvents through the column, from anode to cathode[16]. An integral part of the whole apparatus is

the current power supply, as it applies a voltage, so the motion of particles from anode to cathode can occur. After the analytes have been separated, the detector can identify them[16].
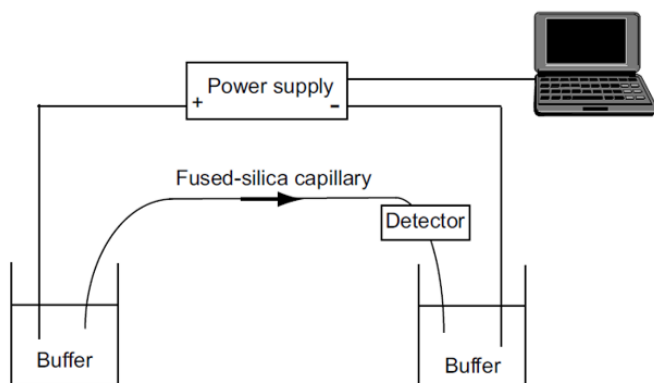
Figure 11: Capillary Electrophoresis.

The whole principle of CE is based on Electrophoretic Mobility and Electroosmotic Flow. Electrophoretic Mobility depends on the solute's and medium's characteristics. More precisely, important parameters are the solute's charge (q), the buffer's viscosity (η), and the solute radius (r), which practically means the size[16]. Temperature affects viscosity as if it is increased, the viscosity decreases[15]. That is why some apparatuses include a temperature control device.

Electroosmotic Flow (Fig. 12) is another principle that governs CE. It is known that the capillary column is made of silica. These silica particles contain silanol groups, are in contact with running buffer, and get de-protonated, release a H^+, and converted into silanate anions, if the buffer has a pH above 3. Cations from the buffer that are near silanates, are attracted by them and make a strong bond, forming the so-called fixed layer. Nevertheless, there is not fully neutralization, which leads more cations to be near this layer than anions. These "latter attracted" cations, make a layer above the fixed layer, the diffuse layer. Both layers together are called the double layer. The cations in the diffuse layer start to flow to the cathode because of opposite charge, also affecting the anions, which normally would move to the anode, but, as cations in this layer are much more than the anions, they "drag" the anions with them and both flow to the cathode[16].
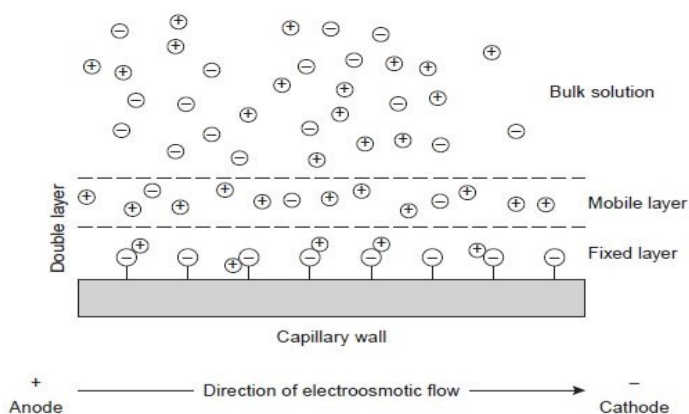
Figure 12: Electroosmotic Flow.

3.5. GAS CHROMATOGRAPHY

Gas Chromatography (Fig. 13) is an analytical method, based on the principle of chromatographic separation. It consists of the stationary phase, which can be either a solid (Gas Solid Chromatography) or a liquid (Gas Liquid Chromatography), and the mobile phase, called carrier gas, which makes this analytical method special. A typical apparatus comprises the following parts: a carrier gas source, the column, an injection port, a detector, and a recording device.
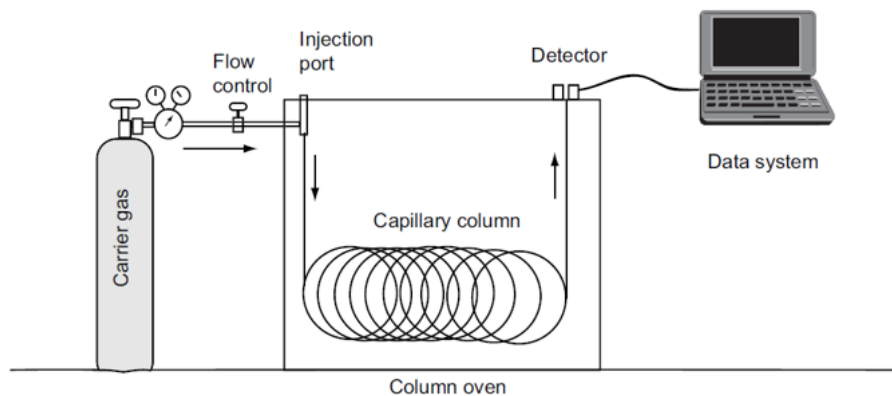
Figure 13: Gas Chromatography.

Carrier gas source is a high-pressure cylinder that contains and provides the carrier gas to the column, with specific pressure and flow rate, after its passage through the valves and the flow meter. Carrier gas must be inert, so it does not react with the stationary phase and the sample. Its role is to carry the intact sample through the column. The main gases used are helium or nitrogen for packed columns and nitrogen, helium, or hydrogen for capillary columns[6]. There are 2 types of columns, the packed column, and the capillary column, with the latter being mostly used. The packed column is 1 to 3

meters long, with an internal diameter of 2 to 4 mm. It is made of glass or metal, and it is packed with silica particles into which the stationary phase is coated. The capillary column is 5 to 60 meters long, having an internal diameter of 0.1 to 0.53 mm[6]. This column is not packed, but inner treated with the stationary phase, which is bonded chemically. There are many different injectors, like syringes, injection valves, or whole automated injection systems. Concerning detectors, flame ionization detectors are a classic option. Last decades, Mass Spectrometry coupled to Gas Chromatography is noticeably used, making it an important detector[17]. Other kinds of detectors for GC are thermal conductivity detectors, electron capture detectors, and nitrogen-phosphorus detectors[12].

The whole principle of GC is based on the retention of gas to the stationary phase. The term distribution constant perfectly describes this, which is equal to the concentration of the volatile compound in the stationary phase to the concentration of this compound in the mobile phase (the carrier gas)[17].

For proper function of the system and suitable results, there is the need for volatile and thermally stable substances. But, many compounds used, contain polar groups, which make them less volatile through intermolecular interactions like hydrogen bonding, more reactive with interfaces, not thermally stable, and susceptible to adsorption. Chromatogram produced may contain peak tailing and not desired results. This drawback is solved through the common step of derivatization of compounds[12].

3.6. DETECTORS

A detector should be able to convert an analyte's response into an electrical signal. Peak areas or peak heights are used for the quantification of an analyte, so its concentration or mass in the mobile phase has to be proportional to this response. Below, 5 main types of detectors will be analyzed, among them are UV, fluorescence, electrochemical, mass spectrometric, and chemiluminescence detection.

3.6.1. UV DETECTION

UV detector is a commonly used detector coupled with HPLC. The main principle is the fact that analytes absorb UV light (in UV or visible region). Such analytes should contain at least one chromophore (to have at least one double bond in structure)[18]. There are 3 main types of UV detectors. The fixed wavelength detector, which is not used

much nowadays, the variable wavelength detector (Fig. 14) and the diode-array detector (DAD). A typical structure of a variable wavelength detector consists of a lamp which commonly is deuterium (or a tungsten lamp in case of visible region) that emits light through a slit. Then, there is the diffraction grating, where light is focused and its function is to spread the light into different wavelengths, which is then rotated to direct a specific wavelength through another slit and the flow cell onto the detector. DAD has almost a similar structure except that light first passes through the flow cell, which is placed before diffraction grating. It is based on an array of diodes, where each diode measures absorbance at a specific wavelength, allowing the simultaneous evaluation of signals at more wavelengths, or even the whole spectra of the analytes within a run[19].
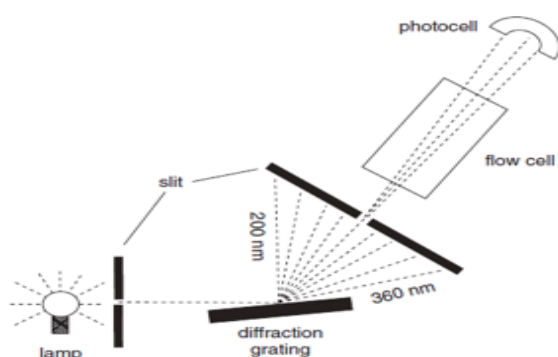
Figure 14: Structure of a variable wavelength UV detector.

3.6.2. FLUORESCENCE DETECTION

Another type is the fluorescence detector (Fig. 15). In this form of detector, compounds analyzed should fluoresce under UV radiation excitation. A typical apparatus consists of a light source that might be deuterium or a xenon flash lamp and spreads light through a filter (in cheaper equipment) or a monochromator (in more expensive equipment). A filter or a monochromator selects the desired excitation wavelength, which is the wavelength used to excite the molecules. They also light the sample while crossing the flow cell. Then, another filter or monochromator selects the wanted emission wavelength (which have a longer wavelength than the excitation wavelength, as there is energy loss during fluorescence) and leads it to the photocell detector, to transform it into an electronic signal[19].
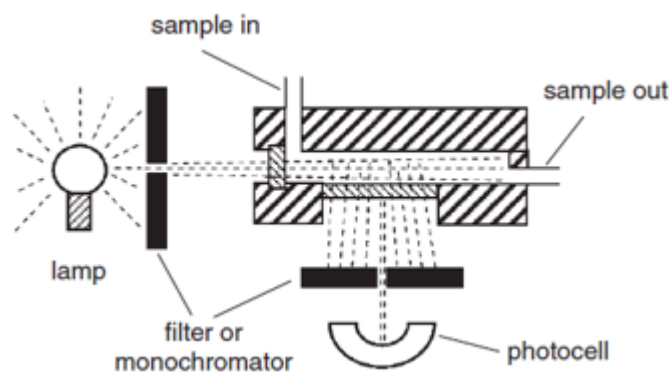
Figure 15: Structure of a fluorescence detector.

3.6.3. ELECTROCHEMICAL DETECTION

The next type and most important detector concerning the analysis of catecholamines is electrochemical detection (Fig. 16). It is based on the reduction or oxidation of compounds and only such compounds with reductive or oxidative properties can be detected. That is why it is the standard method of detection for chemical groups like catecholamines and other neurotransmitters. It consists of 3 electrodes: the working electrode, which comes in contact with the substances and reduction or oxidation reactions occur, the reference electrode, and the auxiliary (counter) electrode. For the actualization of electrochemical reaction on the side of the working electrode, a fixed potential difference between the reference electrode and working electrode takes place. Then, a current is produced from this reaction, which is the output of the detector response, that is stabilized with an opposite current from the working to the counter electrode[20]. This current is linearly proportional to the analyte's concentration, a fact that contributes to quantification[21]. There are many kinds of materials for electrodes, such as carbon, silver, gold, platinum[20], and porous graphite[21], where glassy carbon electrode is the main one commonly used. Two electrochemical modes are mostly used, amperometry and coulometry. They generally differ on the structure of the working electrode, the amount of analyte that reacts with it, and how they react[21]. In amperometry, there is a steady potential applied and conversion efficiency is around 5-10%, as analytes flow over the working electrode[20] but still, sensitivity remains adequate[21]. On the other hand, in coulometry, there are larger surface areas of electrodes and analytes do not flow over the working electrode, but flow through it thus, the conversion efficiency is close to 100%. This fact does not substantially mean that there are better detection limits and less signal-to-noise ratio compared to amperometry, as

background noise also increases, along with the sensitivity. Generally, electrochemical detection offers one of the highest degrees of sensitivity concerning such compounds, while selectivity is of great extent too[21].
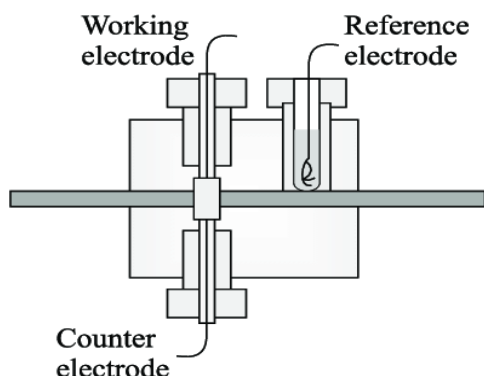
Figure 16: Structure of an electrochemical detector.

3.6.4. CHEMILUMINESCENCE DETECTION

Another notable method is chemiluminescence detection (Fig. 17). When a molecule is able to emit light after a chemical reaction in an excited condition, at a constant temperature, with no production of heat, then one might say that chemiluminescence occurs. There are 2 types of chemiluminescence; direct, where light is emitted by a chemiluminogenic compound, such as luminol, and indirect, in which an intermediate compound is yielded after chemical reaction and transmits its energy to a second compound, able to fluoresce, which in turn emits light. Quantitation is based on the light emitted, which is related to the sum of substances in chemiluminescence reaction[22].
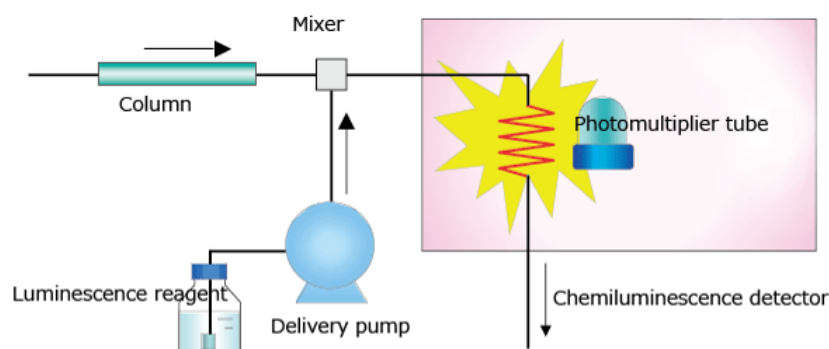
Figure 17: structure of a chemiluminescence detector.

3.6.5. MASS SPECTROMETRY DETECTION

The last detector stated in this paper is Mass Spectrometry (MS) (Fig. 18). It is based on ion production in the gas phase and detection according to the mass-to-charge ratio of

analytes (m/z)[18]. There are 2 main conformations of MS, single-stage detector, also called LC-MS, that detects a single ionic type of an analyte, and multiple reaction monitoring (MRM), or tandem (LC-MS/MS), where isolation of precursor ions, fragmentation into new ions and monitoring of them occurs[19]. The place where analytes get ionized and the mobile phase transits into gaseous phase is called interface, which also connects the HPLC apparatus with MS. There are many types of interfaces, with electrospray interface (ESI) and atmospheric-pressure chemical-ionization interface (APCI) being the most famous. ESI is more common than APCI as degradation is unusual and is favored for polar substances. A disadvantage is degradation that might happen because of high temperature (400-500°C at the interface), but less ionization of matrix has been observed, compared with ESI[19]. Both interfaces can run in positive and negative modes. In positive mode, voltage has a positive polarity and positively charged ions are produced. The opposite is valid for the negative mode[18]. For actualization of this principle, the mobile phase should be made from volatile constituents and buffers[19]. Another important part of an MS system is the mass filter. The leading mass filters are quadrupoles and ion traps. Quadrupole consists of 4 rods, located parallel to each other. They filter according to a selected m/z ratio, through a regulated electric field and then ions move to electron multiplier to be detected. Ion traps are made of a ring electrode with 2 end-cap electrodes. The same principle is applied as for quadrupole filters. Both can create a spectrum of many masses, as they can be controlled to monitor different masses each time. Another mass filter is time-of-flight (TOF) MS, where ions are created in the interface, energy is applied, and forces ions to go through a drift tube of a certain length, with specific velocity. Lighter ions travel faster into the tube than larger ones, under the influence of the same energy. The above 3 mass analyzers concern the single-stage MS. About fragmentation-mode MS, triple quadrupole, and once again, ion trap mass analyzers may be used. After the separation of ions, they should be detected[19]. There are 3 main types of detectors in MS apparatus, among them are electron multiplier and microchannel tube, which both operate according to the impact of ions in an object capable to emit electrons, known as secondary emission. The third type is the Faraday cup, which works according to the collision of an ion with a metal cup[18]. Last thing worth to mention is that single stage has 2 modes: full scan, where whole spectrum of masses is examined in specific time, and selected ion monitoring (SIM), that is chosen in cases where analytes are known and thus scans certain m/z ratios. Results are expressed as intensity of certain mass and limits of detection are much lower

compared to full scan mode, because of greater signal-to-noise ratio. Mass spectrometry is an excellent choice when one wants to identify an analyte in biological material, particularly when concentration is low[18].
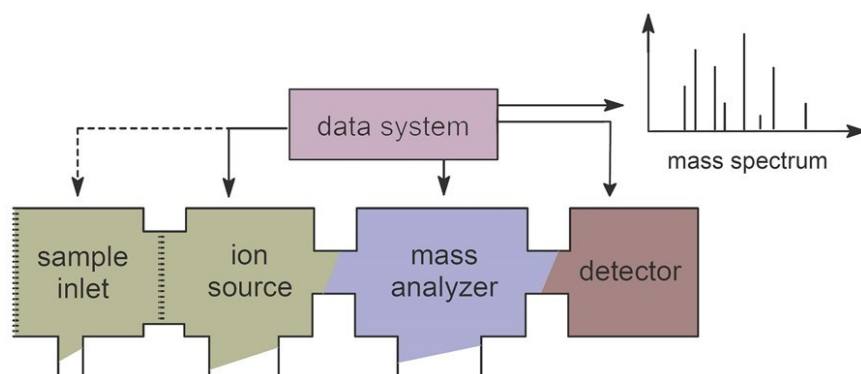
Figure 18: Structure of a Mass Spectrometry detector.

3.7. VALIDATION

Validation is a procedure that establishes that an analytical method is proper to be used for a specific reason and fulfills all the compulsory requirements. Each laboratory must assess validation, to ensure compliance with regulatory authorities. Guideline on bioanalytical method validation from European Medicines Agency (EMA) describes the essential parameters for a well-established validation[23]. Food and Drug Administration (FDA) has published similar guidelines with few differences[24]. A typical validation procedure lies upon certain analytical characteristics, which are *selectivity*, *carry-over*, *(lower) limit of quantification*, *calibration curve*, *accuracy*, *precision*, *dilution integrity*, *matrix effects*, *stability of the analytes in the biological matrix*, and *stability of the analytes and of the internal standard in the stock and working solutions and in extracts during storage and processing*[23].

- *Selectivity* is the ability of the method to distinguish the analytes and the internal standard from the other constituents of the biological material[23].
- *Carry-over* is the presence of an analyte in a blank sample, that follows the analysis of high-concentrated analyte samples[23].
- The lowest amount of analyte that can be quantified accurately and precisely is termed as the *lower limit of quantification (LLOQ or LOQ)*[23].
- *Calibration range* is the range between the lowest and the highest concentration of an analyte for which it has been established that the analytical procedure has

the required degree of accuracy, precision, and linearity. *Calibration curve* is assessed within this range[23]. *Linearity* refers to the method's capacity to produce results that are proportional to the analyte's concentration or amount within this specified range, and it is described by the value of correlation coefficient. Linearity is expressed as the alteration of the regression line's slope, while the range is expressed the same as the concentration units used in the method (e.g., mg/ml, mmol/l)[12].

- *Accuracy* is the closeness of obtained results to the real value[12].

- *Precision* is the degree of agreement between obtained results from repeated measurements of a homogeneous sample. There are 3 classes of precision: repeatability (same analyst, same apparatuses, same day, same laboratory), intermediate precision (same laboratory, different analyst, different equipment, on other days), and reproducibility (different laboratories)[12].

- *Dilution integrity* is used to demonstrate that accuracy and precision are not affected by dilution of the sample[23].

- *Matrix effect* is any direct or indirect change in the response that is caused by unintended analytes or other interfering compounds from the matrix[23].

- *Stability* is the chemical stability of an analyte within a matrix, under specific conditions and a specific time period[23].

Important for the purposes of this paper is a brief description of the *limit of detection* and the *extraction recovery*.

Limit of detection (LOD) is the lowest amount of analyte that can be detected in the sample, but not quantified. Concentration of the analyte is used to express LOD. It is mainly calculated by the S/N ratio, and a suitable value through which the analyte might be detected is either 3:1 or 2:1. In a chromatogram, LOD is the peak with a height at least 2 or 3 times higher than the height of baseline noise. The latter is the same expression as S/N. Similar to LOD, one might use the S/N ratio, which typically is equal to 10:1 in order to quantify the substance (LOQ), and in a chromatogram, the height of the analyte's peak should be at least 10 times higher than the height of baseline noise. There are also other ways to evaluate LOD and LOQ, like the slope of the calibration curve, standard deviation, even visual determination[12].

Extraction recovery in sample preparation is the percentage of analyte retrieved after sample preparation. The equation that characterizes recovery is:

$R = \frac{n_{E,final}}{n_{S,initial}} \cdot 100\%$, or more practical, $R = \frac{C_{E,final} \cdot V_E}{C_{S,initial} \cdot V_S} \cdot 100\%$. Close to 100% or even 100% in some cases is an excellent result. Recovery expresses the extraction efficiency of the method followed, and to assess it, tests including low, medium, and high concentrations are performed, in order to compare results obtained from extracted material with unextracted samples[19].

4. REVIEW PART

The main aim of the Diploma Thesis is to include and study different research papers concerning the evaluation of catecholamines in biological material, using different analytical systems, and to examine how a biological sample should be treated to make this evaluation as reliable as possible. For the comfort of the reader, tables are created, that present each experiment's essential characteristics. The first table demonstrates the different sample preparation methods utilized, the second table exhibits the specific HPLC conditions of every trial, and the third table is an overview of the 3 main analytical methods (HPLC, CE, and GC), able to detect and quantify the catecholamines and their metabolites. In chapter "*Discussion*", the results will be further assessed.

4.1. TABLE FOR SAMPLE PREPARATION

Table 2: Sample preparation methods.

Matrix	Volume (mL)	Sample Preparation	Buffer/ Deproteinization agent (for LLE/depr.)	Elution liquid/Solvent (for SPE/LLE)	Analytes/Recovery (%)	References
blood and urine	blood: NR urine: 20 and then 3 from diluted urine	SPE for blood: Oasis MAX cartridges (=Mixed mode, strong anion exchange) for urine: Bio-Rex 70 cation exchange resin		for blood: 1 mL of 5% acetic acid in methanol for urine: 6 mL of 3.6 mM ammonium pentaborate	NR	25
urine	1	SPE- on 3 ml solid-phase extraction columns packed with reversed-phase octadecylsilane (C18)		1.5 mL of 1 mol/L acetic acid	NR	26
human plasma	0.3	SPE- mixed-mode reversed-phase – strong anion exchange Oasis cartridges (MAX, 30 mg, 1 mL)		1 mL of 5% acetic acid in methanol	absolute recovery MHPG; 92-94 VMA; 91-95	27
mouse urine	NR	modified pre-column switching with PBA-modified pre-column from GL Sciences		20 mmol/L ammonium formate (pH 2.5) and acetonitrile (20/80) (v/v)	NE; 87-99 E; 83-99 DA; 73-86 DHPG; 18-29 DHMA; 79-95	28

Matrix		Method			Recovery	Ref
human plasma	0.05	online SPE- Oasis HLB 30 μm SPE cartridges- Hydrophilic-Lipophilic Balanced RP sorbent		mobile phase A: 10 mM ammonium acetate with 0.1% formic acid mobile phase B: 0.1% formic acid in 100% acetonitrile	NE; 95-105 E; 98-100 DA; 100-104 MN; 95-99 NMN; 97-99 L-DOPA; 97-101 3-MT; 97-100	29
human plasma	0.5	selective SPE on packed fiber solid-phase extraction (PFSPE)columns through polymeric crown ethers-complexation (PCE synthesized in laboratoryof Tianjin Medical University)		50 μL of 12.0 mol/L acetic acid solution	averages of 6 measurements NE; 90.0 E; 60.4 DA; 96.5	30
rat urine	0.2	protein precipitation followed by vortex-mixing with centrifugation	for protein precipitation: 1 mL of methanol containing 1% acetic acid	for vortex-mixing and centrifugation: 50 μL methanol/water(10/90, v/v)	NE; 64.7-79.8 E; 73.9-81.1 DA; 65.0-80.4 MN; 69.2-71.0 NMN; 71.0-82.7 3-MT; 75.5-84.2 DOPAC; 74.2-80.7 DHPG; 67.5-81.0 MHPG; 65.1-76.4 DHMA; 67.0-73.5 VMA; 65.1-73.9 HVA; 68.8-78.3 DOPA; 69.0-74.6 Tyr; 74.1-82.3	31
human urine	0.5	SPE-electro-spun composite fibers functionalized with 4-carboxybenzo-18-crown-6 ether modified XAD resin and polystyrene		1 M acetic acid solution	NE; 70.5 E; 102.8 DA; 119.6	32
human urine	0.1	SPE-polymeric reversed-phase, weak ion exchange mixed- mode sorbent on OASIS®WCX-96 μElution plates		two passes of 25 μL 2% formic acid in 5% aqueous methanol	absolute recovery; 61-96 relative recovery in 3 levels of acidification-catecholamines; 94-111 in no and low acidification, 74-147 in high acidification MN; 94-102 NMN; 95-104	33
rat/mini-pig plasma and rat urine	rat plasma; 0.05 mini-pig plasma; 0.2 rat urine; 0.01	SPE-weak cation exchange on Oasis WCX96-well SPE plates		2 400 μL washes of 5% formic acid in methanol	pig plasma/rat plasma/ rat urine NE; 56.1-65.4/ 66.7-68.9/ 47.9-62.5 E; 63.9-85.1/ 71.5-78.8/ 54.5-63.7 DA; 52.5-60.8/ 79.1-81.3/ 63.5-90.9	34
rat blood micro-dialysate	0.02	SPE-with polymeric ionic liquid (PIL)-diphenylboric acid (DPBA)- packed capillary column			NE; 92.0-96.5 E; 94.0-103.0 DA; 91.0-104.4	35
mouse urine	0.02	SPE- using alumina extraction		200 μL 0.1 N HClO$_4$ containing 0.1 mM sodium metabisulfite	averages recovery in samples; 48 recovery in standard extraction; 60	36
human plasma	1; 0.5 2; 0.5 3; 0.5	1.Protein precipitation 2.SPE- Oasis HLB cartridge 3.SPE- WCX3 cartridges, containing weak ion exchange mixed-mode sorbent	1. 0.5 mL of methanol	2. 4 mL of methanol 3. 500 μL of 0.1% formic acid in acetonitrile and 2 mL of 2% formic acid in acetonitrile	with HLB cartridge MN; 110 NMN; 108	37
rat cortex and hippocampus		deproteinization		0.1 M of HClO$_4$	NE; 88.65-99.82 E; 77.38-99.41 DA; 96.35-99.90 Tyr; 91.23-96.54 MHPG; 86.77-98.70 DOPAC; 73.54-85.79 5-HIAA; 78.71-84.16 HVA; 75.03-83.02 3-MT; 90.68-95.59	38
human urine	1.0	novel aminophenyl- boronic acid functionalized magnetic nanoparticles extraction		1 mL of acidic solution prepared in methanol	NE; 92-106 E; 94-108 DA; 98-104	39

Matrix	Amount	Extraction method	Protein precipitation/reagent	Elution/extraction solvent	Recovery (%)	Ref
human plasma	0.2	SPE- mixed-mode weak cation exchange-on Oasis WCX µElution SPE		60 µL 85% acetonitrile with 1% formic acid	NE; 91.1-103.5 E; 96.2-109.7 DA; 96.4-102.7 MN; 99.7-109.2 NMN; 99.4-106.4 3-MT; 95-8-105	40
human brain	300 mg	protein elimination through filters- 10-kDa Amicon®Ultra Centrifugal Filters			absolute/relative NE; 96.7 ± 0.8/ 96.0 ± 0.5 E; 94.3 ± 2.3/ 94.2 ± 1.2 DA; 97.3 ± 1.8/ 93.2 ± 0.7 MHPG; 99.3 ± 1,0/ 96.5 ± 1.2 DOPAC; 94.3 ± 2.2/ 95.6 ± 0.8 5-HIAA; 100.3 ± 0.6/ 96.4 ± 1.2 HVA; 99.1 ± 1.9/ 96.2 ± 0.3	41
1.human urine 2.human plasma 3.human brain	urine; 1.0 plasma; 0.6	SPE on alumina N columns (and alumina B columns)- ion exchange		5-7 mL of ethyl acetate	NE; 79 ± 5.4 E; 77 ± 4.5 DA; 77 ± 3.3 MN; 82 ± 4.1 NMN; 75 ± 4.3 VMA; 89 ± 5.7 HVA; 77 ± 5.2 MHPG; 81 ± 3.9 DOPAC; 69 ± 3.8 DOPAL; 70 ± 4.0	42
human plasma	0.5	SPE on Waters Oasis HLB cartridge		0.3 mL of methanol (for MHPG extraction) 2. 0.5 mL of mixture of 5% of methanol and 95% of an aqueous solution of 10.5 g/L citric acid, 20 mg/L EDTA and 20 mg/L OSA buffered at pH 3.5 with 1 mol/L NaOH (for CA extraction)	absolute recovery NE; 89-96 E; 85-87 DA; 72-78	43
human plasma	0.5	protein precipitation	1.2 M perchloric acid		DA; 107 DOPAC; 77	44
mouse brain		homogenization, vortex-mixing and centrifugation- for protein precipitation	0.2/ 0.5 mL of deoxyepinephrine		average recovery; 95-100	45
human, rabbit, rat plasma	0.1-0.5	SPE- using alumina extraction		0.04 M phosphoric acid-0.2 M acetic acid (20:80, v:v, pH 1.5–2.0)	NE; 90 ± 8.6, 85 ± 11 E; 72 ± 4.9 and 71 ± 11 DA; 68 ± 12 and 52 ± 6.5 DOPAC; 68 ± 12 and 52 ± 6.5	46
human plasma	0.5	SPE- OASIS HLB (3 types were tested- silica-based C30, MFC18 and polymer-based)		0.5 mL of 2.0% (v/v) acetonitrile and 98.0% (v/v) of aqueous solution (10.5 g/L citric acid and 20 mg/L EDTA 2Na) (pH 2.8)	C30/ MFC18/ PB NE; 87/ 66/ 63 E; 90/ 85/ 90 DA; 87/ 89/ 99 DHPG; 98/ 98/ 68 DOPA; 96/ 92/ 27	47
rat brain	NR	homogenization and Solid Phase Microextraction (SPME) through 4 different fibre coatings		0.5 mL of 0.2 M perchloric acid, EDTA and $Na_2S_2O_5$	NR	48
blended pork	500 mg	SPE- QuEChERS method		12 mL acetonitrile:acetic acid (99:1, v/v), 2.0 gr of $MgSO_4$, 0.5 gr of NaCl	84.5-101.2	49
human urine	4.9	magnet-assisted miniaturized dispersive SPE (boronic acid-modified polyhedral oligomeric silsesquioxanes bound on polydopamine-coated magnetized graphene oxide) Procedures: 1) deproteinization, 2) modified-boronic acid extraction, 3) dissolution and filtration	0.1 mL acetonitrile	1 mL of NH_3-NH_4- buffer (pH 8.5) Dissolution in 0.2 mL of mobile phase (methanol, 10 mM NaH_2PO_4)	E; 88.7-101.7 DA; 81.3-94.9	50
human urine	0.8	protein precipitation	12.4 M perchloric acid		DA; 95.5-101.5 HVA; 92.4-103.6	51
(different regions of) mouse brain	NR	protein precipitation (homogenization and sonication)	homogenization: methanol sonication: 0.2 M perchloric acid		> 90 for all analytes	52
human urine	1.0	SPE- boronate-modified hollow dummy template imprinted polymers- (ASE24 SPE apparatus)		NR	NE; 65.4-99.1 E; 72.7-106.2 DA; 63.4-98.3	53
human urine	0.5	Micro Extraction by Packed Sorbent (MEPS)-		0.1 mL methanol	NE; 93.75-105.77 DA; 93.53-102.01	54

		miniaturized SPE				
rat brain	0.03	Liquid-Liquid Microextraction	0.8 mL of NaHCO$_3$-Na$_2$CO$_3$ buffer (pH 10.5)		NE; 93.9-105 E; 73.9-103 DA; 97.2-103 L-DOPA; 93.9-105 DOPAC; 84.9-93.9 5-HIAA; 95.6-102	55
human plasma	1.0	SPE- on Bond Elut LRC-AccuCAT columns- mixed-mode SPE consisting of strong cation and strong anion exchange sorbent		2 mL mixture of methanol, deionized water, ammonia (30:9:1, v/v/v)	MN; 89.6-99.3 NMN; 88.6-92.7	56
human plasma	0.9	SPE on OASIS MCX-96 well plates- mixed mode, strong cation exchange		0.1 mL of 5% ammonia in methanol	absolute recoveries of pre/post-extraction spiked samples: MN; 90-93 NMN; 74-93 recoveries with IS: MN; 102-103 NMN; 96-104	57
human urine	0.1	SPE- on packed fiber SPE columns with composite nanofibers made of polymeric crown ether with polystyrene		0.05 mL of 12 M acetic acid solution	NE; 92.5-97.6 E; 90.2-94.7 DA; 94.1-95.4	58
human urine	0.2	Simultaneous Extraction Derivatization Pretreatment Procedure (SEDP) with ZnO2		0.3 mL of 1% formic acid in water	NE; 90.7-109.5 E; 91.5-106.8 DA; 91.8-108.7	59
rat brain	NR	homogenization protein precipitation	0.1 mL methanol	2% (v/v) formic acid in methanol	NE; 93.3-100.7 DA; 92.9-98.3 5-HIAA; 89.2-95.5	60
human urine, human plasma	urine; 0.01 plasma; 0.15	Microextraction by packed sorbent (MEPS)- kind of SPE- on 4 mg of solid phase silica C18 sorbent		0.1/ 0.2 of a mixture of methanol and aqueous solution of 30.0 mM citric acid and 0.5 mM sodium 1-octanesulfonate monohydrate (2.5:97.5, v/v) (pH 2.92)	NE; 91.1-95.4 E; 90.0-95.2 DA; 85.1-89.8	61
human urine	5.0	protein precipitation and MEPS- on a magnetic boronic acid modified Ti$_3$C$_2$Tx (Fe$_3$O$_4$@Ti$_3$C$_2$Tx-BA) sorbent	0.1 mL of acetonitrile	0.5 mL of 0.01 M of HCl	NE; 98.56-108.1 E; 92.56-110.0 DA; 98.79-112.3	62
human plasma	0.5	online SPE- on Oasis® weak cation exchange 10 by 1 mm SPE cartridges		A: 100 mM ammonium formate in water (pH 3.0) B: acetonitrile	MN; 97.2-98.9 NMN; 81.6-94.6 3-MT; 90.4-96.6	63
human plasma	0.4	SPE- on Evolute® WCX cartridges (non-polar and weak cation exchange)		0.25 mL acetonitrile containing 2% formic acid	MN; 89.3-111 NMN; 91.2-113	64
human urine	1.0	SPE- on modified SPE cartridge with silica monolith- offline cation exchange		5 mL of 0.1% formic acid	E; 60-67 MN; 99-105 NMN; 55-59	65
human plasma	1.0	SPE- on Oasis HLB 1-mL solid phase cartridges		1 mL of methanol	mean recoveries MN; 96 NMN; 100 mean absolute recoveries MN; 72 NMN; 60	66
human urine	1.0	SPE- on Oasis HLB extraction cartridge		1 mL of 200 mL/L methanol	MN; 99-101.8 NMN; 100.3-106.5	67
human plasma	0.5	SPE- on Strata CW-X extraction cartridges (weak cation mixed mode)		5% formic acid in methanol	MN; 94.6-95.2 NMN; 71.5-80.6	68
sea lamprey brain	for Bond Elut Oasis; 0.5	1. protein precipitation 2. SPE in 3 different cartridges (Bond- Elut C18, Oasis HLB, NEXUS) NEXUS works with non-polar retention mechanism	0.05 mL of 50% acetonitrile/ water (v/v)	for BondElut, Oasis; 1 mL 90% methanol/water for NEXUS; 1 mL of dichloromethane	NE; 69.9-85.7 DA; 82.5-101.5	69
human plasma	0.5	SPE- on Toyopak SP cartridge (cation-exchange)		0.3 mL of 0.6 M potassium chloride-acetonitrile (1:1, v/v)	NE; 72-87 E; 74-85 DA; 85-92	70
human plasma	0.74	dialysis- through a dialyzer block volume of 0.37 mL with a cutoff cuprophan dialysis membrane, and Trace Enrichment Cartridge- with a hema-SB cationic TEC		5 mM diammonium hydrogen orthophosphate (pH 8.3)- as dialysis recipient solvent	absolute recovery NE; 38 ± 2 E; 35 ± 1.5 DA; 19 ± 0.8	71

Matrix	Amount (mL)	Sample preparation	Extraction solvent	Mobile phase / desorption	Recovery (%)	Ref.
human plasma	2.0	protein precipitation	0.45 mL of 1.0 M trichloroacetic acid, 0.05 mL of 5% potassium hydroxide for free compounds 0.3 mL of 9 M $HClO_4$, 0.2 mL of 20% potassium hydroxide for total amount of compounds		free/ total NE; 95.1 ± 1.9/96.0 ± 2.1 E; 95.8 ± 2.3/ 97.4 ± 2.7 DA; 102.4 ± 2.0/ 103.3 ± 1.9 MN; 97.9 ± 2.4/ 96.8 ± 3.1 NMN; 95.3 ± 1.7/ 95.6 ± 1.8	72
human urine and plasma	urine: 0.5 plasma: 0.7	SPE - on Toyopak IC-SP S cartridge (strong cation-exchange resin cartridge)		for urine: 1.5 M of potassium chloride in 100 mM HCl and methanol (93/7, v/v) for plasma: 2 M sodium perchlorate and methanol (93/7, v/v)	from urine samples: L-DOPA: 60 DHPA: 65 HVA: 75 from plasma samples: L-DOPA: 62 DHPA: 65 HVA: 80	73
human urine and plasma	urine: 0.5 plasma: 0.7	SPE - on Toyopak IC-SP S cartridge (strong cation-exchange resin cartridge)		for urine: 2.0 M sodium perchlorate for plasma: 2.0 M sodium perchlorate	from urine samples: L-DOPA: 60 DHBA: 85-90 from plasma samples: L-DOPA: 64 DHBA: 90-93	74
human urine and plasma	urine: 0.03 plasma: 1.5	on-line pretreatment with external carboxymethyl-cellulose-bonded external surface and phenylboronic acid-bonded internal surface (also: for analysis of total catecholamines from urine; acidification with HCl and filtration for plasma; protein precipitation)	for plasma; 90 ml of 60% perchloric acid	the mobile phases for urine and plasma analyses- shown in table "HPLC conditions"	from urine samples: NE; 95 E; 100 DA; 98	75
human urine and plasma	for MHPG: 0.5 ml from urine or plasma for catecholamines: plasma: 1.0 ml/ urine: 0.5 ml	LLE with diphenyl boric acid method for catecholamines/ ethyl acetate extraction for MHPG	for MHPG: ethyl acetate for catecholamines: ammonia buffer (pH 8.7) and heptane with 1% octanol and 0.25%(w/v) TOABr/ octanol and 80 mM acetic acid- for both urine and plasma samples		free MHPG: 76.0 ± 0.36 sulfated MHPG: 71.6 ± 1.01 from urine samples: NE; 99.3 ± 1.98 E; 91.5 ± 1.94 DA; 94.3 ± 1.43 DHBA; 101.2 ± 1.70 from plasma samples: NE; 99.9 ± 2.44 E; 93.4 ± 1.23 DA; 94.6 ± 1.65 DHBA; 100.0 ± 1.58	76
human urine	0.5	deproteinization	1 mM formic acid containing 80% acetonitrile		DA; 89 VMA; 90 5-HIAA; 95	77
human urine	1	SPE-Oasis HLB copolymer SPE sorbent		methanol	quantitation with IS/ES enzymatic hydrolysis: DA; 51, 61/ 31, 35 MN; 120, 134/ 74, 75 NMN; 83, 86/ 46, 53 3-MT; 129, 130/ 69, 83 acidic hydrolysis: DA; 21, 47/ 3, 20 MN; 233, 282/ 80 NMN; 93, 107/ 30, 33 3-MT; 295, 367/ 100, 106	78
human urine	1	SPE-Oasis HLB cartridge		1 ml of methanol	DA; 97.8-109.2 MN; 104.6-124.4 NMN; 103.9-106.1 3-MT; 112.5-122.7	79
human urine	10	SPE- using alumina extraction	10 mL ethyl acetate	2 mL of 0.2 mol/L acetic acid	NE; 76.5-87.3	80
human urine/ human serum	3	Solid Phase Microextraction with monolithic molecularly imprinting polymer (after protein precipitation)	3 mL of acetonitrile	0.18 mL of acetone: acetic acid mixture (7:3, v/v)	urine/serum samples NE; 90,92/ 85,94 E; 86,88/ 88,96 DA; 85,94/ 92,103	81
human urine	10	miniaturized SPE on miniaturized boronate affinity monolithic column		50 mM acetic acid/ methanol (60/40) mixture	92-103	82
artificial urine and human urine	2	MIP-SPE- on "AFFINIMIP Catecholamine and Metanephrine" cartridges		4 mL of methanol/glacial acetic acid (99/1, v/v)	not calculated	83
rat brain	NR	homogenization and protein precipitation	acetonitrile	20 mM phosphate- buffer saline solution (PBS- pH 7.4)	NE; 90.7-103.5 E; 95.4-107.4 DA; 98.0-109.0	84
human urine	0.5	protein precipitation	1 mM formic acid with 20% ACN		3-MT; 93 5-HIAA; 94 VMA; 99	85

rat plasma	NR	protein precipitation	acetonitrile		L-NE; 95 ± 12 L-E; 90 ± 9 DA; 89 ± 9 L-DOPA; 87 ± 9 L-Tyr; 92 ± 7	86
human urine	NR	in-line SPE- on poly(MA-co-EGDMA) SPE monolith-weak cation exchange made in situ		12 mM phosphate and 12 mM sodium ion (pH 3.0)	E; 91.1 ± 2.7 DA; 97.3 ± 1.0 MN; 83.5 ± 0.8	87
human urine	1.0	LLE	1 mL of ethyl acetate-*n*-hexane (8:2 v/v) mixture		E; 108 ± 15.0 DA; 102 ± 1.6 Tyr; 92 ± 1.9	88
human urine	10	SPE- on light alumina B cartridge		1 mL of 0.1 M HCl	NE; 83 ± 4 E; 85 ± 6 DA; 70 ± 3 DOPA; 65 ± 9 DOPAC; 51 ± 14	89
human urine	0.2	LLE and acidification	1 mL of sodium phosphate buffer (pH 7.5), 1 mL dichloromethane, 10% sulfuric acid for acidification/ 3 mL diethyl ether and 2 mL ethyl acetate for extraction		NR	90
human urine	0.1	LLE	0.5 mL ethyl acetate/ 0.1 mL acetonitrile, 0.01 mL methanol, 0.01 mL *N,N*-diisopropylethylamine (and 0.01 mL of 30% volume solution of PFB-Br in acetonitrile for derivatization)		DHPG; 88.3-103.5	91
human urine	5.0	LLE	dilute hydrochloric acid or 5 M sodium hydroxide solution (pH 6.2), 0.1 mL glusulase, 30 mL ethyl acetate with 2% methanol/ 1.5-2.0 mL methanol, ethereal solution of diazoethane, 0.5 mL 2,2 dimethoxypropane		HVA; 98.5 ± 1.2 DOPAC; 96.1 ± 3.4 MHPG; 97.2 ± 1.8 DHPG; 95.4 ± 3.7	92
rat CSF	0.05	protein precipitation	0.15 mL of 50% methanol/ acetonitrile		NR	93
human urine	0.05	Solid Phase Microextraction	polyacrylate 85 μm as fiber coating		NR	94
human plasma	1.0	SPE- using alumina extraction		0.25 mL of 0.1 M acetic acid	DHPG; 56	95
zebrafish larvae eggs	NR	homogenization and protein precipitation	0.35 mL ice-cold methanol		NE-D6; 92 ± 3 E; 96 ± 6 DA-D4; 119 ± 4 NMN; 98 ± 4 3-MT-D4; 95 ± 4	96
human urine	NR	LLE	0.5 mL diluted HCl, 4 g/l of cobalt (II) chloride, NaCl, 2.5 mL of ethyl acetate/ 0.2 ml of hexane		HVA; 75, 97 VMA; 75, 99	97
human urine	2.0	SPE- on Mixed Cation Exchange cartridges		for acidic metabolites; 1 mL of methanol for biogenic amines; 1 mL of 5% NH₄OH in methanol (v/v)	NE; 87.9-107.0 E; 96.9-104.4 DA; 95.3-98.6 NMN; 93.2-104.0 L-DOPA; 92.3-100.4 VMA; 103.9-111.9 DOPAC; 95.2-104.2 HVA; 100.1-107.9 5-HIAA; 94.6-98.6	98
monkey CSF	for unconju-gated/ conjugated DA; 3/ 1	hydrolysis and SPE- using alumina extraction		for hydrolysis; 0.4 M perchloric acid for elution; 2 mL of 0.75 M acetic acid in methanol	for unconjugated DA without/ after hydrolysis; 97.8 ± 8.7/ 98.8 ± 5.4	99
human urine and human plasma	urine; 0.5 plasma; 0.5	LLE	2.0 mL phosphate buffer (pH 7.5), 1.0 mL of carbonate buffer (pH 11)/ 2.0 mL ethyl acetate, 0.1 mL hexane		NE; 95 E; 97	100
rat and dog plasma	0.2	SPE- on phenylboronic acid columns by Varian- acts through a reversible covalent		6 mL of 1 M acetic acid/methanol (6:94, v/v)	NE; 95-112	101

	interaction						
ventral thoracic nerve cords	NR	LLE	1 mL of 1 M aqueous potassium phosphate buffer (pH7.2)/ ethyl acetate, aqueous ammonium hydroxide			NR	102

4.2. TABLE FOR HPLC CONDITIONS

Table 3: HPLC conditions.

Column	IS	Elution	Mobile Phase	Flow Rate (mL/ min)	Detection	Sensitivity (LOD/LOQ)	Refe-rences
PLRP-S 100 Å 5 µm, 150mm×4.6mm ID reversed-phase	none	isocratic	5% acetonitrile, 0.025 M sodium phosphate, 0.025 M citric acid, 0.001 M heptane and sulphonic acid (pH 2.85)	0.75	Optical Fiber coated with enzyme laccase	LOD NE; 20 pM E; 19 pM DA; 19 pM	25
reversed-phase analyticalcolumn Lichrosorb LC- 8, 150 mm × 4.6 mm	none	isocratic	50 mmol/L of potassium dihydrogenphosphate, 100 ml/L of methanol, 200 ml/L of acetonitrile, 500 mg/L of sodium dodecyl sulphate and 250 mg/L of EDTA	1	chemiluminescence	LOD NE; 0.004 µM E; 0.0014 µM DA; 0.005 µM	26
reversed-phase column (Atlantis C_{18}, 150 mm × 4.6 mm I.D.)	3,4-DHBA hydrobromide	isocratic	mixture of methanol (2%, v/v) anda 50 mM citrate buffer (98%, v/v) (pH 3.0)	1	fluorescence	LOD/LOQ MHPG; 0.5/ 1 nM VMA; 1/ 2.5 nM	27
Luna Phenyl-Hexyl 2.0 × 150 mm, 3 µm column (reversed phase fully porous silica)	ascorbic acid in water 0.04% (w/v) containing L-DOPA-d3 (270 nmol/L), DA-d4 (1.7 nmol/L), NE-d6 (3.5 nmol/L), E-d3 (2 nmol/L), 3-MT-d4 (0.7 nmol/L), NMN-d3 (1.2 nmol/L), and MN-d3 (0.7 nmol/L)	gradient	A: 10 mM ammonium acetate with 0.1% formic acid B: 0.1% formic acid in 100% acetonitrile	0.3	MS/MS	LOQ NE; 0.01 nM E; 0.03 nM DA; 0.011 nM 3-MT; 0.01 nM MN; 0.04 nM NMN; 0.05 nM L-DOPA; 1.0 nM	29
Cosmosil 5C$_{18}$ - PAQ	DHBA	isocratic	7.6 g of citric acid, 7.3 g of sodium dihydrogen phosphate, 0.45 g of sodium heptanesulfonate, 0.1 g of EDTA, and 55 mL of acetonitrile (pH 4.2)	1.0	electrochemical	LOD NE; 1.2 nM E; 1.1 nM DA; 1.3 nM	30
Kromasil ODS C_{18}	isoprenaline	gradient	A: methanol B: sodium acetate buffer (pH 5.0)	1.0	fluorescence	LOD NE; 5 nM E; 1 nM DA; 2 nM L-DOPA; 2.5 nM DOPAC; 6.5 nM	103
NR	3 stable isotopes of d$_6$-E, d$_6$- NE hydrochloride and d$_4$-DA hydrochloride	gradient	A: 0.5% HFBA in water B: 0.5% HFBA in methanol	0.6	MS/MS	LOQ for rat and pigplasma; 0.15 nM LOQ for rat urine; 1.48 nM	34
shim-pack XR-ODS III	none	isocratic	mixture of sodium phosphate monobasic dehydrate (75 mM), 1- octanesulfonic acid sodium salt (1.7 mM), 100 µL/L triethylamine (TEA), 25 µM disodium ethylene EDTA, 10% acetonitrile, and KCl (2 mM) in ultrapure water (18.2 MΩ/cm) (pH 3.0)	NR	electrochemical	LOD/ LOQ NE; 0.2/ 0.76 nM E; 0.1/ 0.38 nM DA; 0.2/ 0.65 nM	35

Column	IS/derivatization	Elution	Mobile phase	Flow rate (mL/min)	Detection	LOD/LOQ	Ref
300×5 mm C18 column (BAS model MF-8954)	DHBA	isocratic	sodium acetate (8.204 g), monohydrous citric acid (11.516 g), EDTA disodium salt (0.0584 g), and ProClin solution (10 mL) in 2 L ultrapure water (pH 4.5)	0.4	amperometric	LOD NE; 21.28 pM E; 19.65 pM DA; 23.50 pM	36
pentafluorophenyl stationary phase: Pursuit 3 PFP 150 × 2.0 mm	isotopically labelled IS: MN-d3 and NMN-d3	gradient	(A): 0.1% formic acid in water (B): methanol	0.3	MS/MS	LOQ After PP with ILIS: MN; 0.13 nM NMN; 0.13 nM	37
Hypersil ODS2	vanillic acid	isocratic	10% acetonitrile and a buffer solution (25 mM sodium acetate, 25 mM citric acid, 0.01 mM EDTA- 2Na and 1.0 mM 1-octanesulfonic acid sodium) (pH 3.35)	1.0	amperometric	LOD/LOQ NE; 0.023/ 0.089 μM E; 0.011/ 0.044 μM DA; 0.026/ 0.098 μM DOPAC; 0.012/ 0.048 μM 5-HIAA; 0.013/ 0.039 μM HVA; 0.027/ 0.082 μM 3-MT; 0.036/ 0.12 μM MHPG; 0.11/ 0.33 μM	38
HSS PFP column (UPLC)	DA-d4, E-d6, NE-d6, MN-d3, NMN-d3, and 3-MT-d4	gradient	(A): 0.1% formic acid (B): acetonitrile	0.4	MS/MS	LOD/ LOQ NE; 29.6/ 59.1 pM DA; 13.1/ 32.6 pM E, MN, NMN, 3-MT; 2.7/ 5.5 pM	40
2 microbore ALF-125 columns (250 mm × 1 mm, C18, 3 μm particle size)	DHBA, 5-hydroxy- N-methyl tryptamine oxalate	isocratic	13% methanol combined with a mixture of phosphoric (50 mM) and citric acid (50 mM), octane-1- sulfonic acid sodium salt (1.8 mM), KCl (8 mM) and EDTA (0.1 mM) (pH 3.6)	0.04	electrochemical	LOD/ LOQ NE; 1.2/ 4 pM E; 1.6/ 5.2 pM DA; 2.4/ 8 pM DOPAC; 0.2/ 0.7 pM 5-HIAA; 0.7/ 2.2 pM HVA; 1/ 3.4 pM MHPG; 1.6/ 5.4 pM	41
250×2.0 mm i.d. Capcell Pak SCX UG80 5 μm (strong cation exchanger)	none	isocratic	ammonium acetate buffer (pH 6.0), EDTA, KCl, mixed with methanol (80:20, v/v)	NR	electrochemical	LOD DA; 0.33-0.65 nM NE; 0.3-0.59 nM LOQ NE; 0.59-5.91 nM DA; 0.65-6.53 nM	104
150 mm × 1.5 mm I.D., packed with C18 silica (L- column), from Chemicals Evaluation and Research Institute	none	isocratic	mixture of acetonitrile-15 mM acetate buffer (pH 4.5) (34:66, v/v) containing 1 mM octanesulfonic acid sodium salt	0.1	fluorescence	LOD NE; 4 pM E; 2.5 pM DA; 6.5 pM DHMA; 4.34 pM L-DOPA; 0.28 nM 5-HIAA; 12 pM DOPEG; 11.5 pM DOPAC; 34 pM	105
Varian Microsorb reversed-phase column (C8, 250mm × 4.6mm i.d., 5 μm)	dihydroxyben-zylamine hydrobromide	isocratic	mixture of 5% of methanol and 95% of an aqueous solution of 10.5 g/L citric acid, 20 mg/L EDTA and 20 mg/L OSA buffered at pH 3.5 with 1 mol/L NaOH	1	1. coulometric 2. amperometric	coulometric detection LOD/ LOQ NE; 0.3/ 0.6 nM E; 0.27/ 0.55 nM DA; 0.39/ 0.78 nM amperometric detection NE; 0.6/ 1.2 nM E; 0.55/ 1.1 nM DA; 0.98/ 1.3 nM	43
150mm × 1.5mm i.d.; packed with C18 silica (L-column); particle size 5 μm	none	isocratic	mixture of acetonitrile and 15 mM acetate buffer (pH 4.5) (34:66, v/v) containing 1 mM octanesulfonic acid sodium salt	0.1	fluorescence	LOD NE; 4 pM DA; 6.5 pM 5-HIAA; 12 pM L-DOPA; 0.28 nM DOPAC; 12 pM	106
reversed-phase Hypersil BDS-C18 column	none	gradient	A: 30% of acetonitrile consisting of 30 mM ammonium formate buffer (pH 3.7) B: acetonitrile	1.0	fluorescence and MS/ESI	LOD/ LOQ DA; 1.258/ 2.42 nM 5-HIAA; 0.483/ 2.46 nM	107
150 × 1 mm 5 μm C18 microbore column	none	isocratic	0.1 mM EDTA, 1.0 mM sodium octyl sulfate, 17 mM sodium chloride, and 50 mM sodium phosphate monobasic (pH 4) and 7% v/v acetonitrile	1.1	amperometric	LOD DA; 33-66 pM	108
5 μm Lichrospher 100 RP-18 (Merck)	Deoxyepine-phrine	isocratic	aqueous solution (25 mM potassium dihydrogen phosphate, 0.4 mM heptane sulphonic acid and 50 μM EDTA) and acetonitrile (93–7 v/v)	1.0	dual coulometric	LOD DA; 0.39 nM DOPAC; 0.59 nM 3-MT; 1.08 nM 5-HIAA; 0.42 nM HVA; 0.99 nM	45

Column	Internal standard	Elution	Mobile phase	Flow rate	Detection	LOD/LOQ	Ref
150 mm × 1.0 mm i.d., packed with C18 silica, 5 μm	none	isocratic	mixture of acetonitrile and Briton-Robinson buffer (pH 7.2) (32:68, v/v) (with 5 mM EDTA disodium salt and 5 mM octanesulfonic acid sodium salt)	0.055	fluorescence	LOD NE; 4.2 pM DA; 9.5 pM	[109]
5-mm 250 × 4.6-mm i.d. reversed-phase C18 column (Ultrasphere-ODS)	isoproterenol	isocratic	0.1 M citrate buffer, 0.3 mM EDTA, 0.5 mM sodium 1-octanesulfonic acid in methanol:water (7:93) (pH 2.5)	1.2	coulometric	LOD NE; 11.82 pM E; 10.92 pM DA; 13.06 pM	[46]
150mm × 2.1mm i.d., 3 μm, Discovery C18 HS or a 150mm × 2mm Synergi hydro RP 80 Å, 4 μm	none	gradient	formic acid (0.1%, v/v) in: (A): water, (B): acetonitrile	0.3	MS/MS	LOD with API 3000: NE; 1000 pM DA; 400 pM with API 4000: NE; 600 pM DA; 200 pM	[110]
Model HR-80 column C18	none	isocratic	sodium acetate trihydrate dissolved in polished dH2O, filtered and then addition of sodium dodecyl sulfate, triethylamine, 0.1 M EDTA disodium salt, HPLC grade methanol and HPLC grade acetonitrile (pH 5.6)	1.0	coulometric	LOD NE; 0.12 nM DA; 0.13 nM	[111]
Deverosil RP AQUEOUS -AR-5, packing material: triacontylsilyl silica (C30)- special	DHBA	isocratic	2.0% (v/v) acetonitrile and 98.0% (v/v) of aqueous solution (10.5 g/L citric acid and 20 mg/L EDTA 2Na) (pH 2.8)	0.3	electrochemical	LOD NE; 0.24 nM DHPG; 0.24 nM DOPA; 0.2 nM E; 0.055 nM DA; 0.065 nM LOQ NE; 0.59 nM DHPG; 0.59 nM DOPA; 0.51 nM E; 0.11 nM DA; 0.26 nM	[47]
Capcell Pak C18 UG120	4-methoxytyra-mine	isocratic	75 mM potassium acetate buffer (pH 3.2)/100 mM potassium phosphate buffer (pH 3.2)/acetonitrile (93.1:4.9:2, v/v/v) containing 6 mM sodium 1-hexanesulfonate	0.1	chemiluminescence	LOQ NE; 0.2 nM E; 0.1 nM DA; 0.4 nM MN; 0.15 nM NMN; 0.2 nM 3-MT; 0.4 nM	[112]
Princeton SPHER C18 column	none	isocratic	ion-pairing phosphate- citrate buffer (pH 4.5- containing 65 mg/L OSA) and 6% methanol	0.27	amperometric	LOD/ LOQ DA; 60 pM/ 175 pM	[113]
C8 + Upchurch HPLC column	none	isocratic	citric acid (0.1 M), sodium acetate (0.1 M), OSA (400 mg) and MeOH (15%) (pH 5.5)	0.5	electrochemical	LOD DA; 0.65 nM	[48]
Shim-pack VP-ODS	none	isocratic	0.10 M acetate buffer solution (pH 4.00), 0.42 mM sodium 1-octanesulfonate and 10% (v/v) methanol	1.0	amperometric (wall-jet/ thin-layer)	LOD DA; 1.1 nM HVA; 0.7 nM	[51]
Thermo Scientific Acclaim Trinity P1 column (Nanopolymer Silica Hybrid technology)	none	isocratic	45 mM (NH4)3PO4, 1.1 mM Na4P2O7, and 4% acetonitrile (pH 3.0)	0.65	electrochemical (with boron-doped diamond working electrode)	LOD/ LOQ DA; 0.021/ 0.063 nM	[114]
Luna Omega Polar C18 column	DA-1,1,2,2-d 4 and 5-hydroxyindole-2-carboxylic acid	gradient	0.1% formic acid in: (A): water (B): acetonitrile	0.25	MS/MS	LOD/ LOQ DA, 3-MT; 0.2/ 0.5 nM DOPAC; 50/ 100 nM 5-HIAA; 2/ 5 nM	[115]
C18 MD-150 × 3.2 mm, 3 μm column	DHBA	isocratic	100 mM sodium acetate, 20 mM citric acid, 0.38 mM SOS and 0.15 mM EDTA dissolved in 950 mL polished water and 50 mL acetonitrile (pH 3.3)	0.6	electrochemical	LOD/ LOQ NE; 0.74/ 1.77 nM DA; 0.82/ 1.96 nM DOPAC; 0.74/ 1.78 nM HVA; 0.69/ 1.65 nM 5-HIAA; 0.65/ 1.57 nM	[52]
Thermoscientific C18 column	none	isocratic	MeOH- NaH2PO4 (20 mmol/L, pH 4.0) (5:95, v/v)	0.5	UV	LOD/ LOQ NE; 0.28/ 0.93 μM E; 0.082/ 0.28 μM DA; 0.27/ 0.92 μM	[53]

Column	Internal standard	Elution	Mobile phase	Flow	Detection	LOD/LOQ	Ref
Agilent SB C18 column (UPLC)	isoproterenol hydrochloride and 5-hydroxyindole-2-carboxylic acid	gradient	(A): 5% acetonitrile/ water containing 0.2% formic acid (B): acetonitrile containing 0.2% formic acid	0.2	MS/MS	LOD/ LOQ NE; 0.004/ 0.025 nM E; 0.004/ 0.025 nM DA; 0.002/ 0.015 nM $_L$-DOPA; 0.003/ 0.015 nM DOPAC; 0.003/ 0.025 nM 5-HIAA; 0.008/ 0.04 nM	55
100 mm × 4.6 mm Kinetex 2.6 µm C18 column- UPLC	DHBA	isocratic	0.07 mol/L potassium phosphate, 0.1 mmol/L EDTA, 1.1 mmol/L OSA, 3.1 mmol/L TEA, 14% methanol (pH 3.12)	1.6	electrochemical	LOD NE; 0.8 nM DA; 0.8 nM DOPAC; 0.6 nM HVA; 0.8 nM 3-MT; 1.5 nM 5-HIAA; 0.6 nM	116
Luna® HPLC column (C18 with TMS endcapping)	4-hydroxy-3-methoxybenzyl-lamine	isocratic	NaH_2PO_4 (100 mM), octanesulfonic acid (0.65 mM), EDTA (0.027 mM), acetonitrile (6.7%) (pH 3.35)	1.0	coulometric	LOQ MN; 50.70 pM NMN; 54.59 pM	56
Acquity UPLC® HSS T3 column	3-MT- α,α,β,β-D4 · HCl, $_{DL}$-NMN-α- D2, β-$_{D}$1 · HCl and $_{DL}$-MN-α- D2, β-D1 · HCl	gradient	(A): 0.2% formic acid in water (B): 0.2% formic acid in acetonitrile	0.53	MS/MS	LOQ MN; 0.020 nM NMN; 0.024 nM	57
Sigma HS-F5	none	gradient	A: 0.5% acetic acid water solution B: methanol	0.2	MS/MS	LOD/ LOQ DA; 32.64/ 130.57 pM	117
Acquity HSS T3 column- UPLC	DA-d3	gradient	A: methanol B: 0.1% formic acid solution	0.5	MS/MS	LOD/ LOQ DA; 0.13/ 0.98 nM	118
Waters ACQUITY UPLC ®BEH C18 column	d6-NE	isocratic	water containing 0.1% formic acid and mixture of 0.1% formic acid and acetonitrile (75:25, v/v)	0.3	ESI-MS/MS	LOD/ LOQ NE; 0.3/ 0.89 nM E; 0.19/ 0.55 nM DA; 0.23/ 0.65 nM	59
Inertsil ODS-EP column- UPLC	isoproterenol hydrochloride	gradient	A: 0.01% acetic acid in water B: methanol	1.2	MS/MS	LOD NE; 0.59 nM DA; 0.33 nM 5-HIAA; 0.78 nM LOQ is the lowest value of calibration curve (see *Table for Analytical Methods*)	60
Acquity UPLC BEH C18 column	13C benzoylated or deuterated compounds	gradient	A: 10 mM ammonium formate/0.15% formic acid in water B: acetonitrile	0.3	MS/MS	LOD/ LOQ NE; 0.3/ 1.18 nM E; 0.22/ 0.38 nM DA; 0.13/ 0.98 nM DOPAC; 2.97/ 5.95 nM HVA; 5.49/ 27.45 nM 5-HIAA; 5.23/ 26.15 nM	119
Agilent column UPLC	none	isocratic	methanol and 20 mM acetic acid aqueous solution (15/85, v/v)	1.0	fluorescence	LOD/ LOQ NE; 0.35/ 1.18 nM E; 0.87/ 2.95 nM DA; 0.20/ 0.65 nM	62
Atlantis HILIC Silica column	α,α,β-d3- MN-HCl, α,α,β,β-d4 3-MT- HCl, α,α,β-d3- NMN-HCl	gradient	A: 100 mmol/L ammonium formate in water (pH 3.0) B: acetonitrile	0.4	MS/MS	LOD/ LOQ MN; 0.01/ 0.03 nM NMN; 0.02/ 0.05 nM 3-MT; 0.04/ 0.06 nM	63
Waters Atlantis HILIC silica analytical column UPLC	MN-D3, NMN-D3	gradient	A: water containing 100 mM ammonium formate (pH 3.0) B: acetonitrile	0.3	MS/MS	LOD/ LOQ MN; 0.033/ 0.07 nM NMN; 0.030/ 0.06 nM	64
Phenomenex LUNA Cyano analytical column	d3-NMN- HCl-α,α,β-d3, d3-MN- HCl-α,α,β-d3	isocratic	acetonitrile–water (40/60, v/v) containing 1.5 mmol/L ammonium acetate and 0.6 g/L formic acid	1.5	MS/MS	LOD/ LOQ MN; 0.13/ 0.20 nM NMN; 0.13/ 0.20 nM	66
Discovery RP Amide C16 column	d3-MN, d3-NMN	isocratic	NR	1.0	MS/MS	LOD MN; 0.05 µM NMN; 0.055 µM	67
TSK gel ODS-80TM C18 silica	none	isocratic	15 mM acetate buffer (pH 5.0) and acetonitrile (31%, v/v)	0.05	fluorescence	LOD NE; 26.6 fM E; 57.31 fM	120
microbore column packed with C18 silica (L-column, CERI or TosoHaas)	none	isocratic	15 mM acetate buffer (pH 5.0) and acetonitrile (69:31, v/v) containing 10 mM octanesulfonic acid sodium salt	0.05	fluorescence	LOD NE; 23.64 fM	121
Unison UK C18 column	d3-MN-HCl, d3-NMN-HCl	gradient	A: distilled water containing 0.1% formic acid B: acetonitrile containing 0.1% formic acid	0.3	MS/MS	LOD/ LOQ MN; 0.008/ 0.04 nM NMN; 0.016/ 0.08 nM	68
Waters Symmetry C18 column	$_7$D-melatonin	gradient	A: 1 mM of PFHA in water B: acetonitrile	0.3	MS/MS	LOD/ LOQ NE; 0.83/ 2.84 nM DA; 0.59/ 1.89 nM	69

TSK-gel ODS-120T	Isoproterenol (or N-Methyldopamine if necessary)	isocratic	acetonitrile-methanol-50 mM Tris-hydrochloric acid buffer (pH 7.0) (5:1:4, v/v)	1.0	fluorescence	LOD NE; 0.007 nM E; 0.007 nM DA; 0.01 nM	70
15.0 3 0.46 cm Ultratechsphere 5 mm ODS 2 column	dihydroxyben-zylamine	isocratic	diammonium hydrogen orthophosphate containing heptane sulphonic acid	1.5	coulometric	LOD NE; 0.05 nM E; 0.05 nM	71
Yanapak ODS	o-Tyrosine	gradient	A: phosphate buffer (pH 3.1) B: methanol	0.54	electrochemical and fluorescence	LOD (amperometric/ fluorescence) NE; 0.6/ 3 nM E; 0.5/ 3 nM DA; 1/ 5 nM DOPA; 0.5/ 4 nM MN; 0.8/ 5 nM NMN; 1/ 5 nM	72
TSK- gel ODS 80 TM column	perchloric acid/ isoproterenol/ 3,4 dihydroxyphenyl propanoic acid	isocratic	30 mM citrate buffer (pH 2.5), 10 mM sodium periodate with 3 mM potassium hexacyanoferrrate and aqueous 70% ethanol solution	NR	fluorescence	LOD (urine/ plasma) NE; 0.4/ 0.5 nM E; 0.7/ 0.7 nM DA; 2/ 3 nM L-DOPA; 7/ 9 nM NMN; 0.5/ 0.6 nM MN; 1.3/ 1.5 nM 3-MT; 2/ 3 nM DOPAC; 6/ 4 nM VMA; 69/ - nM HVA; 6/ 4 nM	73
unknown	for urine: 3,4-dihydroxybenzyl amine and 4-hy-droxy-3-metho-xycinnamic acid for plasma: 3,4-dihydroxyben-zylamine	gradient	1) 60 mM citric acid, 32 mM disodium hydrogen phosphate, 1.7 mM sodium hexane sulphonate and 0.1 mM disodium EDTA, 2) solution additionally containing 20% methanol-acetonitrile mixture (3:2, v/v)	NR	fluorescence	LOD (urine/ plasma) NE; 0.6/ 0.6 nM E; 1/ 1 nM DA; 2/ 3 nM NMN; 1/ 1 nM MN; 2/ 3 nM 3-MT; 2/ 3 nM L-DOPA; 12/ 10 nM DOPEG; 2/ 0.5 nM VMA; 300/ 100 nM DOPAC; 7/ 3 nM HVA; 10/ 3 nM	74
for urine: Excelpak SILC8/5C for plasma: Excelpak ICS-C35	DHBA	isocratic	for urine: phosphate buffer, 200 mmol/L potassium nitrate and 0.07 mmol/L EDTA for plasma: citric acid, sodium nitrate and EDTA	for urine: 1.2 for plasma: 1.0	electrochemical	LOQ in urine: NE; 8.9 nM E; 13 nM DA; 20 nM in plasma: NE; 0.06 nM E; 0.082 nM DA; 0.072 nM	75
Spherisorb ODS 2	DHBA for catecholamines	isocratic	Sodium decanesulfonate EDTA, NaH$_2$PO$_4$ and methanol with different composition for catecholamines and MHPG	for MHPG; 0.5 for catecho-lamines; 1.2	electrochemical	LOD NE; 0.5 nM E; 0.8 nM DA; 1.4 nM MHPG; 10 nM	76

4.3. TABLE FOR ANALYTICAL METHODS

Table 4: Overview of analytical methods.

Analytes	Analytical method	Detection	General information	Derivatization	Sensitivity	Recovery (%)	Refe-rences
NE, E, DA	HPLC	Optical Fiber coated with enzyme laccase (special)	sample: blood and urine column: PLRP-S 100 Å 5 µm,150mm×4.6mm ID reversed-phase elution: isocratic flow rate: 0.75 mL/min run time: 7 min	none	LOD NE; 20 pM E; 19 pM DA; 19 pM	NR	25
NE, E, DA	HPLC	chemilumi-nescence	sample: urine column: reversed-phase analytical column Lichrosorb LC-8, 150 mm × 4.6 mm elution: isocratic flow rate: 1.0 mL/min run time: 20 min	none	range NE; 0.03-0.43 µM E; 0.027-0.26 µM DA; 0.033-0.63 µM LOD NE; 0.004 µM E; 0.0014 µM DA; 0.005 µM	NR	26

Analytes	Technique	Detection	Conditions	Derivatization	Range/LOD/LOQ	Recovery	Ref.
MHPG, VMA	HPLC	fluorescence	sample: human plasma column: reversed-phase column (Atlantis C$_{18}$, 150 mm × 4.6 mm I.D.) elution: isocratic flow rate: 1 mL/min run time: 10 min	none	range MHPG; 1-217 nM VMA; 2.5-100 nM LOD/ LOQ MHPG; 0.5/ 1 nM VMA; 1/ 2.5 nM	absolute recovery MHPG; 92-94 VMA; 91-95	27
NE, E, DA,3-MT	HPLC	MS/MS	sample: human plasma column: Luna Phenyl-Hexyl 2.0 × 150 mm, 3 µm column (reversed phase fully porous silica) elution: gradient flow rate: 0.3 mL/min run time: 11.5 min	50 µL of 25% (v/v) propionic anhydride in acetonitrile	LOQ NE; 0.01 nM E; 0.03 nM DA; 0.011 nM 3-MT; 0.01 nM MN; 0.04 nM NMN; 0.05 nM ʟ-DOPA; 1.0 nM	NE; 95-105 E; 98-100 DA; 100-104 MN; 95-99 NMN; 97-99 ʟ-DOPA; 97-101 3-MT; 97-100	29
NE, E, DA	HPLC	electrochemical	sample: human plasma column: Cosmosil 5C$_{18}$ -PAQ elution: isocratic flow rate: 1.0 mL/min	none	range appx. 3-300 nM LOD 1.2 nM	averages of 6 measurements NE; 90.0 E; 60.4 DA; 96.5	30
NE, E, DA, ʟ-DOPA, DOPAC	HPLC	fluorescence	sample: human urine column: Kromasil ODS C18 elution: gradient flow rate: 1.0 mL/min	1,2-diphenylethy-lenediamine	range NE, E, DA, ʟ-DOPA; appx. 0.015-1.100 µM DOPAC; 0.030- 1.19 µM LOD NE; 5 nM E; 1 nM DA; 2 nM ʟ-DOPA; 2.5 nM DOPAC; 6.5 nM	NE; 78.74 E; 86.1 DA; 89.3 ʟ-DOPA; 87.8 DOPAC; 76.76	103
NE, E, DA	UPLC	MS/MS	sample: rat/ mini-pig plasma and rat urine elution: gradient flow rate: 0.6 mL/min	none	range for rat and pig plasma; appx.: 0.6-590 nM range for rat urine; 6-5910 nM LOQ for rat and pig plasma; 0.15 nM LOQ for rat urine; 1.48 nM	pig plasma/rat plasma/ rat urine NE; 56.1-65.4/ 66.7-68.9/ 47.9-62.5 E; 63.9-85.1/ 71.5-78.8/ 54.5-63.7 DA; 52.5-60.8/ 79.1-81.3/ 63.5-90.9	107
NE, E, DA	HPLC	electrochemical	sample: rat blood microdialysate column: shim-pack XR-ODS III elution: isocratic run time: 3.5 min	none	range NE; 0.0018-1.19 µM E; 0.001-1.1 µM DA; 0.0013-1.31 µM LOD/ LOQ NE; 0.2/ 0.77 nM E; 0.11/ 0.38 nM DA; 0.2/ 0.65 nM	NE; 92.0-96.5 E; 94.0-103.0 DA; 91.0-104.4	35
NE, E, DA	HPLC	amperometric	sample: mouse urine column: 300×5 mm C18 column (BAS model MF-8954) elution: isocratic flow rate: 0.4 mL/min	none	LOD NE; 21.28 pM E; 19.65 pM DA; 23.50 pM	averages recovery in samples; 48 recovery in standard extraction; 60	36
MN, NMN	HPLC	MS/MS	sample: human plasma column: pentafluorophe-nyl stationary phase: Pursuit 3 PFP 150 × 2.0 mm elution: gradient flow rate: 0.3 mL/min	dibenzyl-ethoxymethylene malonate (DEEMM)	LOQ after PP: MN; 0.13 nM NMN; 0.13 nM	with HLB cartridge MN; 110 NMN; 108	37
NE, E, DA, DOPAC, 5-HIAA, HVA, 3-MT, MHPG	HPLC	amperometric	sample: rat cortex and hippocampus column: Hypersil ODS2 elution: isocratic flow rate: 1.0 mL/min run time: 20 min	none	range NE; 0.089-3.5 µM E; 0.055-2.2 µM DA; 0.098-2.3 µM DOPAC; 0.06-3 µM 5-HIAA; 00.31-0.8 µM HVA; 00.82-1.4 µM 3-MT; 0.12-1.8 µM MHPG; 0.65-8 µM LOD/ LOQ NE; 0.023/ 0.089 µM E; 0.011/ 0.044 µM DA; 0.026/ 0.098 µM DOPAC; 0.012/ 0.048 µM 5-HIAA; 0.013/ 0.039 µM HVA; 0.027/ 0.082 µM 3-MT; 0.036/ 0.12 µM MHPG; 0.11/ 0.33 µM	NE; 88.65-99.82 E; 77.38-99.41 DA; 96.35-99.90 Tyr; 91.23-96.54 MHPG; 86.77-98.70 DOPAC; 73.54-85.79 5-HIAA; 78.71-84.16 HVA; 75.03-83.02 3-MT; 90.68-95.59	38

Analytes	Technique	Detection	Conditions	Derivatization	Range / LOD / LOQ	Recovery	Ref
NE, E, DA, MN, NMN, 3-MT	UPLC	MS/MS	sample: human plasma column: HSS PFP column elution: gradient flow rate: 0.4 mL/min analytical time: 4 min	none	range NE; 0.059-29.6 nM DA; 0.033-32.6 nM E, MN, NMN, 3-MT; appx.: 0.011-27.3 nM LOD/LOQ NE; 29.6/ 59.1 pM DA; 13.1/ 32.6 pM E, MN, NMN, 3-MT; 2.7/ 5.5 pM	NE; 91.1-103.5 E; 96.2-109.7 DA; 96.4-102.7 MN; 99.7-109.2 NMN; 99.4-106.4 3-MT; 95-8-105	40
NE, E, DA, DOPAC, 5-HIAA, HVA, MHPG	HPLC	electrochemical	sample: human brain column: 2 microbore ALF-125 columns (250 mm × 1 mm, C18, 3 µm particle size) elution: isocratic flow rate: 0.04 mL/min run time: appx. 40 min per sample	none	range NE; 0.24-81.4 nM E; 0.21-138.1 nM DA; 0.27-179.7 nM DOPAC; 0.28-155.5 nM 5-HIAA; 0.22-76.9 nM HVA; 0.58-320.6 nM MHPG; 0.34-225.3 nM LOD/LOQ NE; 1.2/ 4 pM E; 1.6/ 5.2 pM DA; 2.4/ 8 pM DOPAC; 0.2/ 0.7 pM 5-HIAA; 0.7/ 2.2 pM HVA; 1/ 3.4 pM MHPG; 1.6/ 5.4 pM	absolute/relative NE; 96.7 ± 0.8/ 96.0 ± 0.5 E; 94.3 ± 2.3/ 94.2 ± 1.2 DA; 97.3 ± 1.8/ 93.2 ± 0.7 MHPG; 99.3 ± 1,0/ 96.5 ± 1.2 DOPAC; 94.3 ± 2.2/ 95.6 ± 0.8 5-HIAA; 100.3 ± 0.6/ 96.4 ± 1.2 HVA; 99.1 ± 1.9/ 96.2 ± 0.3	41
NE, DA	HPLC	electrochemical	sample: rat brain column: 250×2.0 mm i.d. Capcell Pak SCX UG80 5 µm (strong cation exchanger) elution: isocratic flow rate: 0.0005 mL/min run time: <22 min	none	range DA; 0.33-6.53 nM NE; 0.3-5.9 nM LOD DA; 0.33-0.65 nM NE; 0.3-0.59 nM LOQ NE; 0.59-5.91 nM DA; 0.65-6.53 nM	NR	104
NE, E, DA, DHMA, L- DOPA, 5-HIAA, DOPEG, DOPAC	HPLC	fluorescence	column: 150 mm × 1.5 mm I.D., packed with C18 silica (L-column), from Chemicals Evaluation and Research Institute elution: isocratic flow rate: 0.1 mL/min	1,2-diphenylethy-lenediamine	range up to at least 500 nM LOD NE; 4 pM E; 2.5 pM DA; 6.5 pM DHMA; 4.34 pM L-DOPA; 0.28 nM 5-HIAA; 12 pM DOPEG; 11.5 pM DOPAC; 34 pM	NR	105
NE, E, DA	HPLC	1. coulometric 2. amperometric	sample: human plasma column: Varian Microsorb reversed-phase column (C8, 250mm × 4.6mm i.d., 5 µm) elution: isocratic flow rate: 1.0 mL/min	none	range NE; 1.2-59 nM E; 1.1-54.6 nM DA; 1.3-65.3 nM coulometric detection LOD/ LOQ NE; 0.3/ 0.6 nM E; 0.27/ 0.55 nM DA; 0.39/ 0.78 nM amperometric detection NE; 0.6/ 1.2 nM E; 0.55/ 1.1 nM DA; 0.98/ 1.3 nM	absolute recovery NE; 89-96 E; 85-87 DA; 72-78	43
NE, DA, 5- HIAA, L-DOPA, DOPAC	HPLC	fluorescence	sample: rat brain column: 150mm × 1.5mm i.d.; packed with C18 silica (L-column); particle size 5 µm elution: isocratic flow rate: 0.1 mL/min	benzylamine and then 1,2-diphenylethyle-nediamine	range up to at least 50 nM LOD NE; 4 pM DA; 6.5 pM 5-HIAA; 12 pM L-DOPA; 0.28 nM DOPAC; 12 pM	NE, DA, 5-HIAA, L-DOPA, DOPAC; 94.5-98.5	106
DA, 5-HIAA	HPLC	fluorescence and MS/ESI	sample: rat brain column: reversed-phase Hypersil BDS-C18 column elution: gradient flow rate: 1.0 mL/min	1,2-benzo-3,4-dihydrocarbazole-9-ethyl chloroformate	range 2.44 nM-20 µM LOD/ LOQ DA; 1.258/ 2.42 nM 5-HIAA; 0.483/ 2.46 nM	generally; 91.8-105.6	107
DA	HPLC	amperometric	sample: rat striatum column: 150 × 1 mm 5 µm C18 microbore column elution: isocratic flow rate: 1.1 mL/min run time: 25 min	none	LOD DA; 33- 66 pM	NR	108
DA, DOPAC, 3-MT, 5-HIAA,	HPLC	dual coulometric	sample: mouse brain column: 5 µm Lichrospher 100 RP-18 (Merck) elution: isocratic	none	range DA; 0.196-1.96 µM DOPAC; 0.0594-0.594 µM 3-MT; 0.045-0.45 µM	average recovery; 95-100	45

HVA			flow rate: 1.0 mL/min		5-HIAA; 0.021-0.21 μM HVA; 0.041-0.41 μM LOD DA; 0.39 nM DOPAC; 0.59 nM 3-MT; 1.08 nM 5-HIAA; 0.42 nM HVA; 0.99 nM		
NE, DA	HPLC	fluorescence	sample: rat cortex column: 150 mm × 1.0 mm i.d., packed with C18 silica, 5 μm elution: isocratic flow rate: 0.055 mL/min run time: 60 min	benzylamine and then 1,2-diphenylethy-lenediamine	range up to 50 nM LOD NE; 4.2 pM DA; 9.5 pM	NR	109
NE, E, DA	HPLC	coulometric	sample: human, rabbit, rat plasma column: 5-mm 250 × 4.6-mm i.d. reversed-phase C18 column (Ultrasphere-ODS) elution: isocratic flow rate: 1.2 mL/min	none	range up to 2.36 μM LOD less than: NE; 11.82 pM E; 10.92 pM DA; 13.06 pM	NE; 90 ± 8.6, 85 ± 11 E; 72 ± 4.9 and 71 ± 11 DA; 68 ± 12 and 52 ± 6.5 DOPAC; 68 ± 12 and 52 ± 6.5	46
NE, DA	HPLC	MS/MS	sample: brain dialysates column: 150 mm × 2.1 mm i.d., 3 μm, Discovery C18 HS or a 150mm × 2mm Synergi hydro RP 80 Å, 4 μm elution: gradient flow rate: 0.3 mL/min	none	LOD with API 3000: NE; 1000 pM DA; 400 pM with API 4000: NE; 600 pM DA; 200 pM	NR	110
NE, DA	HPLC	coulometric	sample: mouse nucleus accumbens column: Model HR-80 column C18 elution: isocratic flow rate: 1.0 mL/min run time: 17 min per sample	none	LOD NE; 0.12 nM DA; 0.13 nM	NR	111
NE, E, DA, DOPA, DHPG	HPLC	electrochemical	sample: human plasma column: Deverosil RP AQUEOUS -AR-5, packing material: triacontylsilyl silica (C30) elution: isocratic flow rate: 0.3 mL/min	none	range NE; 0.24-29.6 nM DOPA; 0.2-25.36 nM E; 0.055-10.92 nM DA; 0.065-13.06 nM DHPG; 0.59-29.4 nM LOD/ LOQ NE; 0.24/ 0.59 nM DOPA; 0.2/ 0.51 nM E; 0.055/ 0.11 nM DA; 0.065/ 0.26 nM DHPG; 0.24/ 0.59 nM	C30/ MFC18/ PB NE; 87/ 66/ 63 E; 90/ 85/ 90 DA; 87/ 89/ 99 DHPG; 98/ 98/ 68 DOPA; 96/ 92/ 27	47
NE, E, DA, MN, NMN, 3-MT	HPLC	chemilumi-nescence	sample: mouse plasma column: Capcell Pak C18 UG120 elution: isocratic flow rate: 0.1 mL/min	none	LOQ NE; 0.2 nM E; 0.1 nM DA; 0.4 nM MN; 0.15 nM NMN; 0.2 nM 3-MT; 0.4 nM	NR	112
DA	HPLC	amperometric	sample: rat brain column: Princeton SPHER C18 column elution: isocratic flow rate: 0.27 mL/min run time: 11 min	none	range DA; 0.2-10 nM LOD/ LOQ DA; 60/ 175 pM	NR	113
DA	HPLC	electrochemical	sample: rat brain column: C8 + Upchurch HPLC column elution: isocratic flow rate: 0.5 mL/min	none	LOD DA; 0.65 nM	NR	48
DA, HVA	HPLC	amperometric (wall-jet/ thin-layer)	sample: human urine column: Shim-pack VP-ODS elution: isocratic flow rate: 1.0 mL/min	none	range DA, HVA; 0.01-100 μM LOD DA; 1.1 nM HVA; 0.7 nM	DA; 95.5-101.5 HVA; 92.4-103.6	51
NE, DA, DOPAC, HVA, 3-MT, 5-HIAA	HPLC	electrochemical (with boron-doped diamond working electrode)	sample: mouse brain column: Thermo Scientific Acclaim Trinity P1 column (Nanopolymer Silica	none	range DA; 1-100 nM LOD/ LOQ DA; 0.021/ 0.063 nM	NR	114

48

					range		
			Hybrid technology) elution: isocratic flow rate: 0.65 mL/min run time: for DA, Adenosine;<10 min for more monoamines; 28 min				
DA, 3-MT, DOPAC, 5-HIAA	HPLC	MS/MS	sample: rat brain column: Luna Omega Polar C18 column elution: gradient flow rate: 0.25 mL/min run time: 8.0 min	none	range DA, 3-MT; 0.5-200 nM DOPAC; 100-10000 nM 5-HIAA; 10-1000 nM LOD/ LOQ DA, 3-MT; 0.2/ 0.5 nM DOPAC; 50/ 100 nM 5-HIAA; 2/ 5 nM	NR	115
NE, DA, DOPAC, HVA, 5-HIAA	HPLC	electrochemical	sample: mouse brain column: C18 MD-150 × 3.2 mm, 3 μm column elution: isocratic flow rate: 0.6 mL/min run time: <10 min	none	range NE; 1.77-88.66 nM DA; 1.96-195.85 nM DOPAC; 1.78-89.21 nM HVA; 1.65-82.34 nM 5-HIAA; 1.57-78.46 nM LOD/ LOQ NE; 0.74/ 1.77 nM DA; 0.82/ 1.96 nM DOPAC; 0.74/ 1.78 nM HVA; 0.69/ 1.65 nM 5-HIAA; 0.65/ 1.57 nM	> 90 for all analytes	52
NE, E, DA	HPLC	UV	sample: human urine column: Thermoscientific C18 column elution: isocratic flow rate: 0.5 mL/min	none	range NE; 0.59-59.1 μM E; 0.13-54.6 μM DA; 0.65-65.3 μM LOD/ LOQ NE; 0.28/ 0.93 μM E; 0.082/ 0.28 μM DA; 0.27/ 0.92 μM	NE; 65.4-99.1 E; 72.7-106.2 DA; 63.4-98.3	53
NE, E, DA, L-DOPA, DOPAC, 5-HIAA	UPLC	MS/MS	sample: rat brain column: Agilent SB C18 column elution: gradient flow rate: 0.2 mL/min run time: 7.0 min	Lissamine rhodamine B sulfonylchloride (LRSC)	range NE, E, DA, L-DOPA, DOPAC, 5-HIAA; 0.2-1000 nM LOD/ LOQ NE; 0.004/ 0.025 nM E; 0.004/ 0.025 nM DA; 0.002/ 0.015 nM L-DOPA; 0.003/ 0.015 nM DOPAC; 0.003/ 0.025 nM 5-HIAA; 0.008/ 0.04 nM	NE; 93.9-105 E; 73.9-103 DA; 97.2-103 L-DOPA; 93.9-105 DOPAC; 84.9-93.9 5-HIAA; 95.6-102	55
DA, 5-HIAA, DOPAC, HVA	HPLC	UV-PDA	sample: rat brain column: Hypersil Gold C-18 column elution: isocratic flow rate: 0.2/ 0.25 mL/min	none	range DA; 0.00085-6.53 μM 5-HIAA; 0.00068-5.23 μM DOPAC; 0.017-5.95 μM HVA; 0.015-5.49 μM	DA; 100.5-102.1 DOPAC; 99.2-101.6 5-HIAA; 94.4-98.2 HVA; 95.3-97.7	122
NE, DA, DOPAC, HVA, 3-MT, 5-HIAA	UPLC	electrochemical	column: 100 mm × 4.6 mm Kinetex 2.6 μm C18 column elution: isocratic flow rate: 1.6 mL/min run time: 7.5 min	none	LOD NE; 0.8 nM DA; 0.8 nM DOPAC; 0.6 nM HVA; 0.8 nM 3-MT; 1.5 nM 5-HIAA; 0.6 nM	NR	116
MN, NMN	HPLC	coulometric	sample: human plasma column: Luna® HPLC column (C18 with TMS endcapping) elution: isocratic flow rate: 1.0 mL/min run time: 45 min	none	range MN; 0.051-10.14 nM NMN; 0.055-10.92 nM LOQ MN; 50.70 pM NMN; 54.59 pM	MN; 89.6-99.3 NMN; 88.6-92.7	56
MN, NMN	UPLC	MS/MS	sample: human plasma column: Acquity UPLC® HSS T3 column elution: gradient flow rate: 0.53 mL/min	none	range up to 0.02 nM LOQ MN; 0.020 nM NMN; 0.024 nM	absolute recoveries of pre/post extraction spiked samples: MN; 90-93 NMN; 74-93 recoveries with IS: MN; 102-103 NMN; 96-104	57
DA	HPLC	MS/MS	sample: rat brain column: Sigma HS-F5 elution: gradient flow rate: 0.2 mL/min	none	range DA; 0.13-6.523 nM LOD/ LOQ DA; 32.64/ 130.57 pM	98.2-109.0	117

Analyte	Separation	Detection	Conditions	Derivatization	LOD/LOQ/Range	Recovery	Ref
DA	UPLC	MS/MS	sample: rat brain column: Acquity HSS T3 column elution: gradient flow rate: 0.5 mL/min run time: 2.5 min	none	range DA; 0.98-104.5 nM LOD/ LOQ DA; 0.13/ 0.98 nM	NR	118
NE, E, DA	UPLC	ESI-MS/MS	sample: human urine column: Waters ACQUITY UPLC ®BEH C18 column elution: isocratic flow rate: 0.3 mL/min extraction/ derivatization time: 10 min	phenyl isothiocyanate	range NE; 0.006-1.18 μM E; 0.005-1.10 μM DA; 0.007-1.31 μM LOD/ LOQ NE; 0.3/ 0.89 nM E; 0.19/ 0.55 nM DA; 0.23/ 0.65 nM	NE; 90.7-109.5 E; 91.5-106.8 DA; 91.8-108.7	59
NE, DA, 5-HIAA	UPLC	MS/MS	sample: rat brain column: Inertsil ODS-EP column elution: gradient flow rate: 1.2 mL/min run time: 9.0 min	none	range NE; 5.91- 295.5 μM DA; 6.53-326.4 nM 5-HIAA; 5.23-261.5 nM LOD NE; 0.59 nM DA; 0.33 nM 5-HIAA; 0.78 nM LOQ is the lowest value of calibration curve	NE; 93.3-100.7 DA; 92.9-98.3 5-HIAA; 89.2-95.5	60
NE, E, DA, DOPAC, HVA, 5-HIAA	UPLC	MS/MS	sample: rat CSF column: Acquity UPLC BEH C18 column elution: gradient flow rate: 0.3 mL/min	benzoyl chloride	range NE; 1.18-295.54 nM E; 0.38-272.92 nM DA; 0.98-652.83 nM DOPAC; 0.006-1.19 μM HVA; 0.027-1.10 μM 5-HIAA; 0.026-1.05 μM LOD/ LOQ NE; 0.3/ 1.18 nM E; 0.22/ 0.38 nM DA; 0.13/ 0.98 nM DOPAC; 2.97/ 5.95 nM HVA; 5.49/ 27.45 nM 5-HIAA; 5.23/ 26.15 nM	NR	119
MN, NMN	HPLC	MS/MS	sample: human plasma column: Phenomenex LUNA Cyano analytical column elution: isocratic flow rate: 1.5 mL/min run time: 6.0 min	none	range MN, NMN: 0.20-10 nM LOD/ LOQ MN; 0.13/ 0.20 nM NMN; 0.13/ 0.20 nM	mean recoveries MN; 96 NMN; 100 mean absolute recoveries MN; 72 NMN; 60	66
NE, E, DA	HPLC	electrochemical	sample: rat adrenal gland column: ISRP-C18, Phenomenex, luna 5 μm elution: isocratic run time: 12 min	none	LOD NE; 2.96 nM E; 2.73 nM DA; 3.26 nM	NR	123
MN, NMN	HPLC	MS/MS	sample: human urine column: Discovery RP Amide C16 column flow rate: 1.0 mL/min run time: 3.0 min	none	range MN; 0.05-25.35 μM NMN; 0.055-27.29 μM LOD MN; 0.05 μM NMN; 0.055 μM	MN; 99-101.8 NMN; 100.3-106.5	67
NE, E	HPLC	fluorescence	sample: rat brain column: TSK gel ODS-80TM C18 silica elution: isocratic flow rate: 0.05 mL/min run time: 30 min	benzylamine (solution)	range NE; up to 59.11 pM E; up to 54.58 pM LOD NE; 26.6 fM E; 57.31 fM	NR	120
NE	HPLC	fluorescence	sample: mouse brain column: microbore column packed with C18 silica (L-column, CERI or TosoHaas) elution: isocratic flow rate: 0.05 mL/min	benzylamine	LOD NE; 23.64 fM	NR	121
MN, NMN	HPLC	MS/MS	sample: human plasma column: Unison UK C18 column elution: gradient flow rate: 0.3 mL/min run time: 5.0 min	none	range MN; 0.04-50 nM NMN; 0.08-100 nM LOD/ LOQ MN; 0.008/ 0.04 nM NMN; 0.016/ 0.08 nM	MN; 94.6-95.2 NMN; 71.5-80.6	68

Analytes	Technique	Detection	Conditions	Derivatization	Range / LOD / LOQ	Recovery (%)	Ref
NE, DA	HPLC	MS/MS	sample: sea lamprey brain column: Waters Symmetry C18 column elution: gradient flow rate: 0.3 mL/min run time: 10 min	none	range NE; 2.96-591 nM DA; 3.26-652.8 nM LOD/ LOQ NE; 0.83/ 2.84 nM DA; 0.59/ 1.89 nM	NE; 69.9-85.7 DA; 82.5-101.5	69
NE, E, DA	UPLC	fluorescence	sample: human urine column: Agilent column elution: isocratic flow rate: 1.0 mL/min	none	range NE; 5.91 nM-2.96 µM E; 5.46 nM-2.73 µM DA; 6.53 nM-3.26 µM LOD/ LOQ NE; 0.35/ 1.18 nM E; 0.87/ 2.95 nM DA; 0.20/ 0.65 nM	NE; 98.56-108.1 E; 92.56-110.0 DA; 98.79-112.3	62
MN, NMN, 3-MT	HILIC	MS/MS	sample: human plasma column: Atlantis HILIC Silica column elution: gradient flow rate: 0.4 mL/min run time: 8.0 min	none	range MN, NMN, 3-MT; 0-20 nM LOD/ LOQ MN; 0.01/ 0.03 nM NMN; 0.02/ 0.05 nM 3-MT; 0.04/ 0.06 nM	MN; 97.2-98.9 NMN; 81.6-94.6 3-MT; 90.4-96.6	63
MN, NMN	UPLC	MS/MS	sample: human plasma column: Waters Atlantis HILIC silica analytical column elution: gradient flow rate: 0.3 mL/min	none	LOD/ LOQ MN; 0.033/ 0.07 nM NMN; 0.030/ 0.06 nM	MN; 89.3-111 NMN; 91.2-113	64
NE, E, DA	HPLC	fluorescence	sample: human plasma column: TSK-gel ODS-120T elution: isocratic flow rate: 1 mL/min run time: 8 min	1,2-diphenylethylene-diamine	range 0.04-20 nM LOD NE; 0.007 nM E; 0.007 nM DA; 0.01 nM	NE: 65.1 E: 62.8 DA: 79.8 (averages)	70
NE, E, DA	HPLC	coulometric	sample: human plasma column: Ultratechsphere 5 mm ODS 2 column elution: isocratic flow rate: 1.5 mL/min	none	LOD NE; 0.05 nM E; 0.05 nM	absolute recovery NE: 38 ± 2 E: 35 ± 1.5 DA: 19 ± 0.8	71
NE, E, DA, NMN, MN, 3-O-methyl-DOPA, 3-O-methyl-DA, o-Tyrosine	HPLC	electrochemical and fluorescence	sample: human plasma column: Yanapak ODS elution: gradient flow rate: 0.54 mL/min	none	range appx. 1.48-591.1 nM with ED appx. 5.9-591.1 nM with FD LOD (amperometric/ fluorescence) NE; 0.6/ 3 nM E; 0.5/ 3 nM DA; 1/ 5 nM DOPA; 0.5/ 4 nM MN; 0.8/ 5 nM NMN; 1/ 5 nM	NE: 96.0 ± 2.1 E: 97.4 ± 2.7 DA: 103.3 ± 1.9 NMN: 95.6 ± 1.8 MN: 96.8 ± 3.1 3-O-methyl-DOPA: 98.4 ± 1.2 3-O-methyl-DA: 95.3 ± 2.4	72
L-DOPA, DHPA, HVA	HPLC	fluorescence	sample: human urine and plasma column: TSK- gel ODS 80 Tₘ column elution: isocratic	periodate	range (urine) L-DOPA; 4 nM-1(2) µM VMA; 20 nM-6 µM LOD (urine/plasma) NE; 0.4/ 0.5 nM E; 0.7/ 0.7 nM DA; 2/ 3 nM L-DOPA; 7/ 9 nM NMN; 0.5/ 0.6 nM MN; 1.3/ 1.5 nM 3-MT; 2/ 3 nM DOPAC; 6/ 4 nM VMA; 69/ - nM HVA; 6/ 4 nM	from urine samples: L-DOPA: 60 DHPA: 65 HVA: 75 from plasma samples: L-DOPA: 62 DHPA: 65 HVA: 80	73
L-DOPA, DHBA	HPLC	fluorescence	sample: human urine and plasma elution: gradient	1,2-diphenylethylene-diamine	range L-DOPA; 4 nM-1(2) µM VMA; 20 nM-1(6) µM LOD (urine/ plasma) NE; 0.6/ 0.6 nM E; 1/ 1 nM DA; 2/ 3 nM NMN; 1/ 1 nM MN; 2/ 3 nM 3-MT; 2/ 3 nM L-DOPA; 12/ 10 nM DOPEG; 2/ 0.5 nM VMA; 300/ 100 nM DOPAC; 7/ 3 nM HVA; 10/ 3 nM	from urine samples: L-DOPA: 60 DHBA: 85-90 from plasma samples: L-DOPA: 64 DHBA: 90-93	74

Analytes	Method	Detection	Conditions	Derivatization	LOD/LOQ/range	Recovery	Ref.
NE, E, DA	HPLC	electrochemical	sample: human urine and plasma column: for urine: Excelpak SILC8/5C for plasma: Excelpak ICS-C35 elution: isocratic flow rate (urine/plasma): 1.2/1.0 mL/min	none	LOQ (urine/ plasma) NE; 8.9/ 0.06 nM E; 13/ 0.082 nM DA; 20/ 0.072 nM	from urine samples: NE; 95 E; 100 DA; 98	75
NE, E, DA, MHPG	HPLC	electrochemical	sample: human urine and plasma column: Spherisorb ODS 2 elution: isocratic flow rate: 0.5 mL/min for MHPG, 1.2 mL/min for catecholamines	none	range (plasma/ urine) NE, E, DA; 0.75-15/ 100-1000 nM MHPG; 1-4/ 1-6 nM LOD NE; 0.5 nM E; 0.8 nM DA; 1.4 nM MHPG; 10 nM	free MHPG: 76.0 ± 0.36 sulfated MHPG: 71.6 ± 1.01 from urine samples: NE; 99.3 ± 1.98 E; 91.5 ± 1.94 DA; 94.3 ± 1.43 DHBA; 101.2 ± 1.70 from plasma samples: NE; 99.9 ± 2.44 E; 93.4 ± 1.23 DA; 94.6 ± 1.65 DHBA; 100.0 ± 1.58	76
DA, MN, VMA, 5-HIAA, 3-MT, HMBA, DHBA	CE	UV	sample: human urine capillary: fused-silica run time: 33 min buffer: poly (diallyldime-thylammonium chloride) (PDDAC)	none	LOD DA; 105.5 nM MN; 80.6 nM VMA; 122.1 nM 5-HIAA; 17.8 nM 3-MT; 92.1 nM HMBA; 39.2 nM DHBA; 80.5 nM	DA; 89 VMA; 90 5-HIAA; 95	77
NE, E, DA, MN, NMN, 3-MT, DHBA, HMBA	CE	1. UV 2. MS	sample: human urine capillary: fused-silica run time: 18 min buffer: 1. 20 mM ammonium acetate 2. 10 mM ammonium acetate	none	LOD between different solvents (CE-UV/ CE-MS) NE; 0.86-2.15/ 0.83 μM E; 0.67-1.96/ 1.27 μM DA; 1.14-2.39/ 1.30-1.90 μM MN; 0.76-1.92/ 1.05-3.15 μM NMN; 0.91-1.29/ 0.89-3.17 μM 3-MT; 0.69-1.56/ 0.78-1.14 μM DHBA; 0.76-1.72/ 0.48 μM HMBA; 0.50-0.89/ 0.53-4.12 μM	NR	124
DA, MN, NMN, 3-MT	CE	1. UV 2. MS	sample: human urine run time: 18-23 min total buffer: 50 mM ammonium acetate (pH 4.0)	none	LOD (CE-UV/ CE-MS) DA; 0.7/ 1.2 μM MN; 0.8/ 1.4 μM NMN; 0.5/ 0.7 μM 3-MT; 0.5/ 0.9 μM	quantitation with IS/ES enzymatic hydrolysis: DA; 51, 61/ 31, 35 MN; 120, 134/ 74, 75 NMN; 83, 86/ 46, 53 3-MT; 129, 130/ 69, 83 acidic hydrolysis: DA; 21, 47/ 3, 20 MN; 233, 282/ 80 NMN; 93, 107/ 30, 33 3-MT; 295, 367/ 100, 106	78
DA, MN, NMN, 3-MT, HMBA	CE	UV	sample: human urine buffer: 50 mM ammonium acetate–40 mM diisopropylamine (pH 4.0)	none	LOD/ LOQ DA; 0.70/ 0.83 μM MN; 0.40/ 0.50 μM NMN; 0.57/ 0.87 μM 3-MT; 0.53/ 0.67 μM HMBA; 0.40/ 0.49 μM	DA; 97.8-109.2 MN; 104.6-124.4 NMN; 103.9-106.1 3-MT; 112.5-122.7	79
NE	CE	electrochemilumi-nescence	sample: human urine capillary: fused-silica buffer: 20 mmol/L PBS containing 4 mmol/L SDS added with an amount of TPA (pH 8.0)	none	range: 0.07-20 μM LOD NE; 26 nM LOQ NE; 0.26 μM	NE; 76.5-87.3	80
NE, E, DA	CE	laser-induced fluorescence	sample: rat pheochromo-cytoma tumor cells capillary: fused-silica MT <18 min buffer: 40 mmol/L sodium tetraborate and 60 mmol/L boric acid mixture (pH 9.0)	fluorescein isothiocyanate	range; 0.05-1.0 μM LOD NE; 0.15 nM E; 0.31 nM DA; 0.08 nM	NE; 97.16 ± 0.97 DA; 91.00 ± 0.49	125
NE, E, DA	CE	amperometric	sample: human urine capillary: fused-silica buffer: 0.15 M MES (pH 5.57)	none	LOD NE; 0.16 nM E; 0.14 nM DA; 0.41 nM	NE; 102.6 E; 106.7 DA; 90.1	126

NE, E, DA	CE	electrochemical	sample: human urine capillary: fused-silica buffer: 160 mmol/L sodium phosphate	none	range NE, E, DA; 1-25 nM LOD NE; 0.6 nM E; 0.522 nM DA; 0.442 nM	NE; 102 E; 110 DA; 106	127
E	CE	chemilumi-nescence	sample: human urine capillary: uncoated fused-silica buffer: 3 M phosphate buffer (pH 11.2)	none	LOD E; 6.9 nM	97.4-102.4	128
NE	CE	electrochemical	sample: rat heart, spleen, small intestine capillary: fused-silica buffer: 250 mM borate (pH 8.8)	none	range NE; 80-1000 nM LOD NE; 68.1 ± 19.0 nM	NE; 95.1 ± 5.6	129
DA	CE	UV	sample: human urine capillary: fused-silica buffer: BGE, 30 mM NaOH, 0.1% HPC, pH 6.5 (adjusted with MES)	none	range DA; 0-10 μM LOD DA; 0.075 μM	NR	130
NE, E, DA	CE	fluorescence	sample: rat brain MT: NE; 7.8 min E; 7.5 min DA; 8.0 min buffer: boric acid and borax (various concentrations)	FITC	LOD NE; 0.14 pM E; 0.19 pM DA; 0.14 pM	normal injection/ FASS mode NE; 76.0/ - E; 85.2/ - DA; 83.7/ 102.7	131
NE, E, DA	CE	amperometric	sample: rat brain capillary: fused-silica buffer: 0.18 mol/L PBS (pH 5.8)	none	range NE; 1.0-100 nM E; 0.9-100 nM DA; 0.75-100 nM LOD NE; 0.92 nM E; 0.74 nM DA; 0.49 nM	NE; 90.7-103.5 E; 95.4-107.4 DA; 98.0-109.0	84
DA, NMN, 5-HIAA, VMA, 3-MT, HMBA	CE	UV	sample: human urine capillary: fused-silica buffer: 500 mM Tris-borate (TB, pH 9)	none	LOD DA; 204.2 nM NMN; 154.4 nM 5-HIAA; 30.3 nM VMA; 313.4 nM 3-MT; 98.7 nM HMBA; 131.6 nM	3-MT; 93 5-HIAA; 94 VMA; 99	85
L-NE, L-E, DA, L-DOPA, L-Tyr	CE	MS/MS with ESI (after UV detection)	sample: rat plasma capillary: uncoated fused-silica buffer: 2 M formic acid (pH 1.2)	none	LOD/ LOQ L-NE; 150/ 500 nM L-E; 40/ 133 nM DA; 77/ 257 nM L-DOPA; 54/ 180 nM L-Tyr; 67/ 223 nM	L-NE; 95 ± 12 L-E; 90 ± 9 DA; 89 ± 9 L-DOPA; 87 ± 9 L-Tyr; 92 ± 7	86
DA	CE	FSCV	sample: Drosophila Larva brain capillary: fused-silica buffer: 200 mM NaH_2PO_4 with 1 mM tetraborate (pH 4.5)	none	LOD DA; around 1 nM	NR	132
DA	CE	fluorescence	sample: rat brain capillary: uncoated fused-silica run time: <20 min buffer: 15 mM borate buffer (pH 10.2)	fluoresceine-5-isothiocyanate (FITC)	range DA; 0.001-0.3 μM LOD DA; 0.10 nM	DA; 94.1	133
E, DA, MN	CE	UV	sample: human urine capillary: fused-silica buffer: 6 mM phosphate buffer (pH 7)/ 12 mM phosphate buffer (pH 3)	γ-methacryloxy-propyltrime-thoxysilane (capillary derivatization)	LOD E; 23.47 nM DA; 24.15 nM MN; 14.70 nM	E; 91.1 ± 2.7 DA; 97.3 ± 1.0 MN; 83.5 ± 0.8	87
NE, DA	CE	laser-induced fluorescence	sample: rat striatum capillary: fused-silica buffer: 110 mM phosphate buffer (pH 7.05 ± 0.02)	naphthalene-2,3-dicarboxaldehy-de	LOD NE; 0.14 nM DA; 0.25 nM	NR	134
NE, E, DA, MN, NMN, DOPA, HVA,	CE	fluorescence	sample: bovine adrenal medullary cells capillary: fused-silica buffer: 0.1 M boric acid (pH 5.7)	none	LOD NE; 90 nM E; 100 nM DA; 80 nM MN; 25 nM	NR	135

Analytes	Technique	Detection	Sample/Column conditions	Reagent	LOD/LOQ/range	Recovery	Ref
DOPAC					NMN; 5 nM DOPA; 80 nM HVA; 90 nM DOPAC; 8 nM		
NE, E, DA, DOPA, DOPAC	CE	fluorescence detection (sensitized luminescence of terbium ions)	sample: urine capillary: fused-silica buffer: 30 mM boric acid (pH 10)	none	LOD NE; 0.08 µM E; 0.07 µM DA; 0.13 µM DOPA; 0.09 µM DOPAC; 0.1 µM	NE: 83 ± 4 E: 85 ± 6 DA: 70 ± 3 DOPA: 65 ± 9 DOPAC: 51 ± 14	89
NE, E, DA, DHBA, DOPAC, NMN, MN, VMA, HVA, 5-HIAA, 3-MT	CE	photodiode array (PDA) (UV)	sample: human urine capillary used: fused-silica buffer: for cationic compounds; acetic acid buffer (pH 4.0) for anionic compounds, phosphate-tetraborate buffer (pH 8.0)	none	range 10-200 nM LOD 5 nM LOQ NMN, MN, DOPAC, 5-HIAA, HVA, 3-MT; 10 nM	NR	136
DA, L-DOPA	GC	MS/MS	sample: rat CSF column: DB-5MS 30 m × 0.25 mm I.D. × 0.25 µm film thickness carrier gas: helium flow rate: 1.2 mL/min run time: 15-20 min	2,2,3,3,3-pentafluoro-1-propanol and pentafluoropropionic acetic anhydride	range DA; 0.33-424.3 nM L-DOPA; 0.25-329.6 nM	NR	93
NE, E, DA, HVA, DOPAC, NMN, VMA, DOPA	GC	MS	sample: human urine, blood, plasma column: DB-5MS capillary column carrier gas: helium flow rate: 1.0 mL/min	MSTFA, MTBSTFA, BSTFA, BSTFA with 1% TMCS, TMSI, MBTFA, TFAA, MBHFBA	range/ LOD HVA; 5.49 nM-27.45 µM/ 1.10 nM DA; 65.28 nM-32.64 µM/ 16.32 nM DOPAC; 29.74 nM-29.74 µM/ 2.97 nM NMN; 27.29 nM-27.29 µM/ 8.19 nM VMA; 5.05 nM-25.23 µM/ 1.01 nM NE; 118.22 nM-29.55 µM/ 29.55 nM E; 109.17 nM-27.29 µM/ 27.29 nM DOPA; 25.36 nM-25.36 µM/ 5.07 nM	NR	137
NE, DA	GC	triple quadrupole MS	sample: human urine column: Thermo TR-5MS carrier gas: helium flow rate: 1.0 mL/min	100 µL propyl chloroformate, 100 µL pyridine	LOD/ LOQ NE; 0.08/ 0.13 µM DA; 0.004/ 0.005 µM	NR	138
HVA, VMA, 5-HIAA	GC	triple quadrupole MS	sample: human urine column: Thermo TR-5MS carrier gas: helium flow rate: 1.0 mL/min	ethyl chloroformate/ ethanol	range appx. 0.003-0.52 mM LOD/ LOQ HVA; 0.007/ 0.015 µM VMA; 0.0002/ 0.0003 µM 5-HIAA; 0.13/ 0.26 µM	NR	94
DHPG	GC	MS	sample: human plasma column: fused-silica capillary column with cross-linked DB-17 stationary phase	tert-butyldimethylchlorosilane/ imidazole	range DHPG; 3-29 nM LOD DHPG; 0.1 nM	DHPG; 56	95
NE, E, DA, NMN, 3-MT	GC	MS	sample: zebrafish larvae eggs column: J&W DB-5 carrier gas: helium flow rate: 1.0 mL/min	hexamethyldisilazane (HMDS), N-methyl-bis-heptafluorobutyramide (MBHFBA)	LOD/ LOQ NE; 4.73/ 15.96 nM E; 152.8/ 518.5 nM DA; 4.57/ 16.32 nM NMN; 2.18/ 8.19 nM 3-MT; 2.39/ 7.18 nM	NE-D$_6$; 92 ± 3 E; 96 ± 6 DA-D$_4$; 119 ± 4 NMN; 98 ± 4 3-MT-D$_4$; 95 ± 4	96
HVA, VMA	GC	electron impact MS	sample: human urine column: DB-5MS fused-silica capillary column carrier gas: helium run time: 30 min	N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA)	LOD HVA; 10.98 nM VMA; 5.05 nM	HVA; 75, 97 VMA; 75, 99	97
NE, E, DA, NMN, HVA, DOPAC, VMA, L-DOPA, 5-HIAA	GC	MS	sample: human urine column: DB-5MS capillary column carrier gas: helium flow rate: 1.0 mL/min	hexamethyldisilazane (HMDS), N-methyl-bis (heptafluorobutyramide) (MBHFBA)	range/ LOD/ LOQ NE; 59.11 nM-5.91 µM/ 6.80 nM/ 32.39 nM E; 109.2 nM-13.65 µM/ 34.06 nM/ 97.38 nM DA; 65.28 nM-6.53 µM/ 9.27 nM/ 41.00 nM NMN; 54.59 nM-5.46 µM/ 5.02 nM/ 15.56 nM	NE; 87.9-107.0 E; 96.9-104.4 DA; 95.3-98.6 NMN; 93.2-104.0 L-DOPA; 92.3-100.4 VMA; 103.9-111.9 DOPAC; 95.2-104.2 HVA; 100.1-107.9 5-HIAA; 94.6-98.6	98

					HVA; 5.49 nM-10.98 μM/ 1.48 nM/ 4.12 nM DOPAC; 5.95 nM-11.89 μM/ 0.71 nM/ 4.04 nM VMA; 5.05 nM-10.09 μM/ 0.25 nM/ 0.86 nM L-DOPA; 101.4 nM-12.68 μM/ 22.01 nM/ 70.39 nM 5-HIAA; 5.23 nM-10.46 μM/ 0.37 nM/ 1.41 nM	
DA	GC	MS	sample: monkey CSF column: glass column packed with 3% OV 17 on 100/ 120 mesh Supelcoport flow rate: 15 mL/min	trifluoroacetic anhydride	LOD DA; appx. 0.3 nM	for unconjugated DA without/ after hydrolysis; 97.8 ± 8.7/ 98.8 ± 5.4	99
HVA, DOPAC, VMA, 5-HIAA	GC	MS	sample: human urine column: Ultra-2 cross-linked capillary column carrier gas: helium flow rate: 0.5 mL/min	ECF, MTBSTFA +1% *tert*-butyldimethyl-chlorosilane	range appx. 0.27-27.45 μM LOD HVA; 0.005 nM DOPAC; 27.95 nM VMA; 0.10 nM 5-HIAA; 0.10 nM	NR	90
DHPG	GC	MS/MS	sample: human urine column:DB-5ht fused silica column	PFB-Br, BSTFA	LOD DHPG; 76 pM	DHPG; 88.3-103.5	91
HVA, DOPAC, MHPG, DHPG	GC	flame ionization detection	sample: human urine column: U-shaped glass column packed with 3% OV-101 carrier gas: nitrogen flow rate: 30 mL/min	*n*-butylboronic acid, diazoethane	range HVA; 0.02-0.09 μM DOPAC; 0.02-0.10 μM MHPG; 0.02-0.09 μM DHPG; 0.02-0.09 μM	HVA; 98.5 ± 1.2 DOPAC; 96.1 ± 3.4 MHPG; 97.2 ± 1.8 DHPG; 95.4 ± 3.7	92
NE, E, MN, NMN	GC	MS	sample: human urine and plasma column: fused-silica capillary column carrier gas: helium flow rate: 28 cm/sec	methyl chloroformate	LOD NE; 0.3 nM E; 0.27 nM DA; 0.33 nM	NE: 99.50 E: 99.65	100
NE	GC	MS/MS with negative ion chemical ionization	sample: rat and dog plasma column: Restek capillary column carrier gas: argon	PFPA–ethyl acetate (1:2, v/v)	range 0.06-11.82 nM LOQ 0.06 nM	in the SPE effluent: 58 ± 2.3 after solvent removal and deriva-tization: 37 ± 8.8 in final sample: 19 ± 2.6	101

5. DISCUSSION

5.1. SAMPLE PREPARATION

5.1.1. FOR HPLC

Before an HPLC detection, it is important for the sample to be appropriately treated. In the articles mentioned, SPE appears in more than half of them, making this approach the most used. Specific preference of an SPE mode is not observed, as reversed-phase mode, ion-exchange mode, and mixed-mode show up nearly equally, maybe ion-exchange stands out. As stated in the theoretical part, there is the possibility of choosing among different solvents, according to the type of SPE. In these analyses, methanol is widely applied, confirming the theory that it is the most used solvent. To a high extent, formic acid, acetonitrile, and acetic acid are suitable for conducting the experiments as

well. One might observe that in many cases, not a single solvent is used, but a combination of them. This benefits the breakage of both hydrophobic and ionic bonds of the analytes to the stationary phase.

Besides the classical SPE columns and techniques, in the included articles special SPE sample preparations took place. First, an SPE column was packed with polymeric crown ethers containing dibenzo-18-crown-6 subunit and polystyrene nanofibers (PFSPE with PCE-PS), for selective extraction of plasma CAs. Recoveries were great for NE and DA, but for E were quite low (60.4%)[30]. This method was based on a previous study from the same analysts, where a PBA-SPE cartridge was used to create a PFSPE column with PCE-PS composite nanofibers, and diphenylborinic acid (DPBA) as a complexing agent. Satisfactory absolute recoveries were obtained with this technique[58]. Both methods are characterized by the short analytical time needed, less consumed volumes, and simplicity. In the same manner, other analysts used modified SPE columns with composite fibers of 4-carboxybenzo-18-crown-6 ether modified XAD resin and polystyrene, which provided better recovery values compared to the previous method, except NE which was extracted up to 70.5%. This method is also cheap, as it did not contain much amount of solvent, adsorbent, and volume of sample[32]. Another subtype of SPE was introduced for the determination of DA in pork tissue, the QuEChERS method, which stands for quick, easy, cheap, effective, rugged, and safe. It gave recoveries between 84.5 to 101.2, making it a successful method[49]. Scientists tried to create a hollow dummy template imprinted boronate-modified polymer (B-hDIPs) packed SPE column, which have advantages, like high affinity to the *cis*-diol groups and that they contain holes in their arrangement, which give the possibility of removing templates and quick mass transfer. It provides satisfactory recoveries, but selectivity needs improvement, and it is a time-consuming production[44].

The next more common sample preparation is PP. Different deproteinization agents appear in the articles, in which perchloric acid and methanol are the dominant ones, turning up in half of PPs each. Formic acid and acetonitrile can also be spotted, among others.

On the other hand, LLE is not used as much as the two previous procedures. Whenever used, analysts took advantage of ammonia-(ammonium) buffer, with pH values around 8.7, as a common buffer, and heptane as an extraction solvent.

Apart from the 3 common sample preparation methods, scientists came up with a few novel procedures. One of them was to purify the sample in a PBA pre-column included in the HPLC apparatus directly and analyze it through a HILIC system later. This extraction offers high selectivity, suitability, and reproducibility, with acceptable recoveries, except DHPG[28]. Another method used aminophenylboronic acid functionalized magnetic nanoparticles (Fe$_3$O$_4$@APBA), which present great affinity to the *cis*-diol group of catecholamines, thus selectivity, with high recoveries. Interesting is that these particles can be recycled for up to 5 extractions, with no significant loss of recovery values and so, reducing the costs[39]. Moreover, quick extraction of catecholamines was accomplished by boronic acid-modified polyhedral oligomeric silsesquioxanes on polydopamine-coated magnetized graphene oxide. This adsorbent offers high sensitivity and selectivity for CAs among the different compounds in urine, because of its high adsorption capacity for ortho-phenols. As for Fe$_3$O$_4$@APBA, they can be reused as well, with an insignificant reduction of the recovery[50]. Finally, remarkable was the dialysis of a plasma sample, followed by trace enrichment with a strong cation-exchange resin trace enrichment cartridge (TEC), which allowed a fully automated analysis using ASTED.XL™ for a sensitive, economical, and precise routine assay of free plasma catecholamines. The dialyzer and the TEC may be applicable for a period of 6 months. However, absolute recoveries were not so satisfying[71].

5.1.2. FOR CE

CE is an analytical method that may be conducted without previous sample preparation. This is justified also by the findings of this DT, where more than half of CE analyses did not use any of the known preparation procedures. Among those that did, it is noted that SPE was the method of choice (most of these articles used SPE). As in HPLC, there is not a favorite kind of SPE, and different modes were tested. Methanol is the most suitable solvent once again, along with acetic acid of various concentrations, mostly in mixtures.

There are some special SPE methods worth mentioning, one of which is the in-line miniaturized boronate affinity monolithic column (μBAMC) that is fully automated and able to preconcentrate catecholamines from a small amount of urine in a relatively short time, with high recoveries[82]. Another in-line SPE principle is the utilization of an SPE column composed of poly (MA-co-EGDMA) monolith, which makes easier the transfer

from the SPE segment to the separation zone of the capillary, improves sensitivity, and lowers LOD, compared to other conventional approaches[87]. Lastly, molecular imprinted polymer SPE (MIP-SPE) can remove salts from urine, giving the opportunity of high sensitivity when working with field-amplified sample injection CE (FASI-CE) for determination of CAs in human urine[83].

PP is the second most common sample preparation method in the articles studied. In contrast to PP in HPLC, here acetonitrile is the deproteinization agent of choice and not perchloric acid, which, interestingly, was never used.

LLE seems to be an unfavorable method for CE, as it is met only once. That is reasonable, as catecholamines are hydrophilic compounds, and it is difficult to find an organic solvent immiscible with water and concurrently able to dissolve the compounds of interest. Briefly, LLE is suitable for the extraction of more lipophilic substances. Also, organic compounds from samples may be extracted along with the wanted analytes and interfere with CE, producing poor results[139]. The extraction solvent used in this single LLE was ethyl acetate-hexane.

5.1.3. FOR GC

In GC, SPE is not the most used method but appears equally with LLE. Probably because GC is not a common analytical method nowadays for the detection of catecholamines and belongs to past times, where LLE was more widespread. As in previous methods, different SPE modes take place and the preferred solvents for the elution of catecholamines are methanol, and acetic acid of several concentrations, either alone or in mixtures.

LLE seems to be the preparation of choice, in contrast to HPLC and CE. A suitable buffer is (potassium) phosphate, with a pH range around 7.5. As an extraction solvent, ethyl acetate was mostly used, followed by hexane.

The least used sample preparation technique in GC is PP. It is not as favored as in HPLC and CE. From the articles reviewed, no preference for a specific deproteinization agent is displayed, as methanol and acetonitrile are equally presented in the experiments.

5.2. RESULTS AND OVERVIEW OF HPLC ANALYSIS

HPLC is the analytical method of choice for the detection of catecholamines and their metabolites. Many articles and experiments were studied in this DT. Among them, the HPLC system was connected to many types of detectors, like different kinds of MS, electrochemical detectors (both amperometric and coulometric), fluorescent detectors, chemiluminescent detectors, even UV detectors. A special type of detection also appeared, where HPLC was coupled to an optical fiber analyzer coated with the enzyme laccase[25]. To figure out which type of detection provides the best results, sensitivity is the main thing to be considered. This of course does not depend only on the detector, but on other factors as well, specified later. There were 25 procedures that gave the lowest sensitivities (LOD, LOQ, and range). 10 of them used MS detectors, another 9 took advantage of electrochemical detectors and fluorescence detection was applied in the rest 6 articles, making them the most suitable detectors for HPLC analyses. Detection with the optical fiber also gave very low results and it may be considered an appropriate, special analyzer. More accurately, these procedures can sense catecholamines and their metabolites up to a LOD in the low pM range. Concerning the elution type, isocratic elution was utilized more than gradient, which is about the 1/3. In addition, 9 of the 25 previously declared methods operated under gradient conditions, and 16 of them under isocratic. On the other hand, when the polarity of the compounds to be analyzed are similar, isocratic elution may provide better resolution and that is why isocratic elution is more used, as catecholamines do have similar polarities. There is also the need to investigate the relation of the 2 elution types with the run times of the whole HPLC (and UPLC) process. Gradient elution in HPLC offered the ability to perform fast analysis in less than 8 minutes, with average run times among 10 to 15 minutes. In UPLC, the analysis was done quickly, ranging from 2.5 minutes to 9 minutes. On the other hand, when using isocratic elution in HPLC, the lowest run times were between 3 to 8 minutes, the average around 15 to 20 minutes, and some high runs from 40 to 60 minutes. Isocratic elution was used 3 times in UPLC, providing a run time of 7.5 minutes in one of them. This is a proof that gradient elution can decrease the run times of the analysis and may be preferred in such cases.

From the above 25 studies, one might want to find out which among them were done the quickest, besides having the lowest sensitivity values, and to become aware of their

characteristics. 8 of them gave both high sensitivity and fast analysis. HPLC was used 5 times and UPLC 3 times. Isocratic and gradient elution were performed equally, 4 times each. About detection, MS/MS was the most common (appeared 4 times), followed by electrochemical detection (2 times), optical fiber analyzer, and fluorescence detection (1 time each). This conclusion leads to the fact that UPLC begins to take the lead over a common HPLC apparatus, since it runs much quicker, may provide low LOD values, even better than HPLC, consumes fewer solvents, and produces better peak separation and sharper peaks. Nevertheless, HPLC has been established as the main analytical apparatus and every laboratory has one. The only advantage of an HPLC system over a UPLC may be the economical part. About elution, both, if well operated, can provide sensitive outcomes. Isocratic may have the benefit of simplicity and low cost over a gradient, as there is the consumption of one kind of mobile phase, and that is why it is widely used in cases of similar analytes. Finally, even though HPLC coupled to ED is the conventional analysis for catecholamines, MS/MS is proved to be highly sensitive, making it an ideal detector too. Fluorescence detection can be suitable as well, but the need for derivatization most of the time expands the run time, compared to ED and MS. Speaking of fluorescent derivatization, out of 13 studies including fluorescence detection, approximately 1/5 tried to conduct the analysis without any derivatization, using only the native fluorescence of the compounds. Between the reports where derivatization occurred, diphenylethylenediamine was the most common agent, which was used 6 times. Besides diphenylethylenediamine, many derivatization agents were described, like benzylamine, periodate, and 1,2-benzo-3,4-dihydrocarbazole-9-ethyl chloroformate.

Let's look more thoroughly at each type of compound studied. As there is a huge number of articles concerning the detection of catecholamines with HPLC or UPLC, there was the possibility to make improved categorizations. If the studies able to give the lowest sensitivity values are taken as standards, one can make important conclusions, about the origin of the sample, elution, detection, analysis times, and sensitivity. Some articles managed to determine only the 3 main catecholamines, DA, NE, and E. Samples were taken from plasma, urine, and brain, without preference to one of them. About elution, isocratic was preferred over gradient elution, as it is present 4 times more. Electrochemical detection was present 5 times from 15 in total, as was fluorescence (5 times), and lastly, MS/MS (3 times). As mentioned in the beginning,

LODs were in the range of 0.1-10 pM and LOQs around 0.01 nM. The lowest detection limit was achieved in a certain case, reaching 26 fM[120]. Other authors described procedures to detect specifically the metanephrines, MN and NMN. Interestingly, all samples were taken from human plasma and gradient elution was the dominant type (4/6 studies). Almost all of them (5/6) operated with an MS/MS detector and sensitivity was great once again, accomplishing low pM (around 10 pM). Thus, for metanephrine determination, superior conditions are clear. Dopamine detection alone was also of high interest. It is noted that only brain samples were taken for analysis. Isocratic elution was mostly (3/4 researches) employed, along with electrochemical (3/4) detection. LOD once again was close to pM level (average around 30 pM). Finally, studies were carried out to analyze different metabolites of catecholamines, with or without catecholamines themselves. Such metabolites are VMA, MHPG, DOPAC, 5-HIAA, HVA, 3-MT, DHPG, and DOPA. For this category, studies apart from the "top 25" were under consideration, as the latter only dealt with the 3 basic catecholamines and metanephrines. The main organ used for sampling was the brain, followed by the plasma and the urine. Isocratic elution was used in 7 from 10 papers, and concerning the detection, electrochemical detection (6/10), MS (3/10), and fluorescence (2/10, in one paper along with MS) were employed, in that order. Detection limits were higher than in the 25 articles, around 0.2 nM for DHPG and DOPA and 0.5-1 nM for the rest of the metabolites.

5.3. RESULTS AND OVERVIEW OF CE ANALYSIS

CE is another suitable analytical method widely applied for the determination of the compounds of interest in this DT. Experiments took place using different detection modes, like UV, fluorescence, electrochemical detection, MS, chemiluminescence, even fast-scan cyclic voltammetry in one special case. The situation here is different compared to HPLC, as the lowest sensitivity values could be obtained by electrochemical and fluorescent detections. Generally, one might spot that the detectors employed more frequently in a CE system are electrochemical, fluorescence, and UV. Considering run times (or migration times when run time is not stated in the article), a range between 18 to 23 minutes (average around 20 minutes) is typical when conducting a CE experiment. The fast time of analysis in CE can be also attributed to the fact that in more than half of the studies, sample preparation is not needed, so less

time is consumed. In a CE apparatus, a significant role has the buffer. The most used ones are phosphate and borate buffers (around 20 times each), followed by acetic acid/acetate buffers and formic acid buffers (8 and 3 times, respectively). Another statistical data that needs to be declared is the derivatization in cases of fluorescence. This detection shows up in 6 studies, from which native fluorescence was used 2 times. FITC and NDA were the most popular derivatization agents.

It is necessary to look at this method more closely, according to the type of compounds analyzed. About the 3 main catecholamines only, the best sensitivities could be obtained, with an average LOD range around 0.1-0.5 nM, and a lower value in a report of 0.14 pM. Samples were drawn from human urine, rat brain, and in one case rat pheochromocytoma tumor cells. The detectors of choice were electrochemical and fluorescence and run times were within the expected average. In other reports, scientists managed to identify DA alone from human urine and brain samples. Here, no detection technique seems to be favored, as electrochemical, UV, and fluorescence are utilized once each. The lowest LODs observed are 0.1 nM, 1 nM, and 75 nM. No studies were done to detect the metanephrines only, but together with catecholamines and metabolites, so the next category contains all kinds of compounds. Urine has the advantage as the suitable origin for a sample, from which material was taken 8 times from 10 cases. Regarding the detection method, UV was applied 7 times and it is the common detector here, followed by MS (applied 3 times). Lastly, the mean LOD value was 0.1 μM for these compounds-MN, NMN, 3-MT, DHBA, HMBA, VMA, 5-HIAA, L-DOPA, L-Tyrosine, and DOPAC. There were exceptions with lower LODs, around 5 nM for MN, NMN, DOPAC, 5-HIAA, HVA, and 3-MT.

5.4. RESULTS AND OVERVIEW OF GC ANALYSIS

Gas Chromatography is the final analytical method evaluated in this DT for the identification and detection of catecholamines and their metabolites. A remarkable fact is that, as GC's main principle is the analysis of volatile compounds and catecholamines are not naturally much volatile, derivatization must take place for this procedure to be successful. If one examines the articles included about GC or looks at the corresponding table, he will notice many different derivatization agents. The ones that stood out were *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA- 3/14), hexamethyldisilazane (HMDS- 2/14), trifluoroacetic anhydride (TFAA- 2/14), *N*-methyl-

bis(heptafluorobutyramide) (MBHFBA- 2/14), and *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA- 1/14). Pentafluoropropionic anhydride (PFPA) was widely used as well. This signifies that (trimethyl)silylation (with BSTFA, HMDS, MSTFA) and acylation (with TFAA, MBHFBA, PFPA) are suitable derivatizations for polar compounds, such as catecholamines and their products. This mandatory step makes GC time-consuming and complex. As in the previous two analytical methods, detection should be investigated. Of all the 14 studies, MS was the most employed one, showed in 13 of them. The rest of the detectors were flame ionization detectors and electron capture detectors[140],[141] (in articles not included in tables), which are exclusive detectors to the GC process. In GC, mobile phases do not have the same sense as in HPLC but are called carrier gases, as they are in the gaseous phase. The most used one was helium (9/14), followed by argon (1/14), nitrogen (1/14), methane (in mixtures with argon)[141], and hydrogen[140] (methane and hydrogen in articles not included in tables). About run times, articles often do not state how long the operation took place and so, a thoughtful conclusion cannot be made. In a few of them, it is mentioned that run time was 15-20 minutes. Yet, considering the derivatization and the sample preparation that are required, one assumes that the period before the GC procedure is extensive. Let's look at these articles more thoroughly. Classes that could be created according to the desired results are the articles that researched for the 3 main catecholamines along with other metabolites and the articles that studied only the products of catecholamines. About the first case, samples were taken more times from urine and plasma, equally. MS/MS was the detector of choice, as expected, and the more frequent carrier gas employed was helium. Lastly, average LODs were on the scale of 0.25 nM-2.4 nM for NE, E, DA, HVA, DOPAC, NMN, VMA, DOPA, MHPG, 5-HIAA, and 3-MT. In an article[100], LOD for the 3 catecholamines was up to 0.3 nM. In another study[101], the lowest value of LOQ could be reached for NE, at 0.06 nM, in which argon was again used, a thing that illustrates that argon may not be much utilized but provides high sensitivities. In the second category, samples were mostly taken from human urine, and in all of them, MS was the detection mode. Helium once again was applied commonly and mean LODs were around 0.1-0.2 nM for HVA, VMA, 5-HIAA, and DHPG. Better values for HVA[90] and DHPG[91] were observed, 5 pM and 76 pM respectively.

5.5. COMPARISON OF ANALYTICAL METHODS

As the 3 basic analytical methods have been discussed in detail, there must be a discussion about their advantages and disadvantages, especially in comparison to one another. The basic parameters to characterize the most suitable procedure, such as total consumed time and sensitivity should be kept in mind during this point. Let's begin with the least used technique, the GC. This method, generally speaking, has high efficiency because of the high temperatures and volatility and may offer quicker separation than HPLC. What one could observe in the previous analysis is the fact that in all cases, derivatization should exist for the proper analysis, otherwise, the hydrophilic and polar substances of interest would not be detected, or the analysis time would be large enough. When the identification was accomplished by an HPLC or a CE apparatus, derivatization was not needed, except in cases where fluorescence detection was employed and that was not always true, or in other more special instances. Immediately, GC becomes more time-consuming and by keeping in mind its sensitivity values, it is less preferred than the other 2. Next, should be the comparison between CE and HPLC. In the latter, usually, the sample should be adequately prepared to extract the substances from the complex biological material and then, inject them into the HPLC system. On the other hand, in CE, in more than half of the situations studied, this is not an issue, as sample preparation is not necessary and organic material can be directly introduced into the apparatus. Additionally, CE is operated easier and has a simpler structure than an HPLC system. The analysis time depends on many other factors as well, like the mobile phase (in HPLC), the buffer (in CE), the carrier gas (in GC), the flow rate, and broadly, on the conditions chosen. Nevertheless, if one wants to make a general conclusion about which analytical method provides the fastest results, that would be the CE.

Another parameter that should be evaluated and compared between the 3 methods is the sensitivity of each apparatus, expressed through LOD, LOQ, and/or range. Concerning the GC, it could detect the catecholamines and their products in an average of 0.1 nM to 2.5 nM. CE provided a sensitivity of the 3 catecholamines around 0.1-0.5 nM and for the rest of the compounds around 0.1 μM. About HPLC, this system can give values up to 0.1-10 pM and is undoubtfully the most sensitive method, a fact that explains why it is also the widest used one for the detection of such substances.

An important factor is also the possibility of detecting isolated compounds. In GC, very few articles were found that could detect a compound separately from others. Almost all experiments detect compounds together with other catecholamines or other metabolites. Either this is not possible with GC or analysts were not interested in it. In CE studies, there were efforts to selectively identify DA, E, or NE, which is an advantage, indicating that CE may be used to specify only one substance through a matrix with other similar ones. LODs were also satisfactory, between 0.1-75 nM. Analysts also tried to determine molecules with an HPLC system. Presented articles include detection of DA mainly, and to a less extent, NE. LODs for DA were very low, around 30 pM, and for NE[121] 23.64 fM. HPLC articles were double the amount of CE articles, however articles attempted to examine just one compound were approximately equal, thus no conclusion can be made for which method is more suitable to accomplish this.

It would be helpful to mention other general benefits of each system, to create a more spherical look around this subject. It is assured that low sensitivities are the main thing analysts attempt to achieve. But sometimes, it would be of greater importance for the procedure to be simple, especially when inexperienced analysts or students handle the apparatuses, and to be cheaper, for similar occasions or when the budget for laboratory equipment is low. An HPLC apparatus is more complex than GC and CE, more solvents are used, the equipment is expensive, such as the pumps, and an experienced analyst should operate it. GC may be cheaper than HPLC in terms of equipment and maintenance, but high temperatures arise which could be dangerous, and detections are mainly destructive. On the other hand, CE does not demand an extensive number of solvents as an HPLC, and instrumentation is simpler. A disadvantage is the high voltage that is applied. So, if one wants an undemanding apparatus for the detection of catecholamines, CE could be a great choice with sufficient sensitivity. Nevertheless, for major research, HPLC is the suitable system to be applied, as it is the "standard" apparatus presented in every laboratory, providing the lowest LODs. Also, it can be easily automated, a thing that decreases the run times and improves precision and reproducibility[142].

6. CONCLUSION

Catecholamines from biological material were analyzed with 3 main systems, HPLC, CE, and GC. Many conclusions could be drawn through the included articles. Sample

preparation is an integrated part of HPLC and GC, but not so much in CE. Between the 3 main sample preparation procedures (SPE, LLE, and PP) SPE was the most applied, as it gives high extraction recoveries, with methanol as a solvent. In GC, LLE is chosen as much as SPE. Phosphate buffer with a pH around 7.5 and ethyl acetate as extraction solvent are widely selected. Besides how a biological material should be handled for appropriate analysis, this DT researched the suitable conditions for the determination of the desired substances. In general, the lowest concentrations of catecholamines, up to a pM range, could be detected with an HPLC-MS or HPLC-ED apparatus. The mobile phase was pumped in the system more through isocratic elution, rather than gradient elution, but that does not denote that isocratic elution performs better, as gradient elution can contribute to low sensitivity values with shorter run times. In recent years, UPLC starts to prevail over HPLC, because it is faster and more sensitive than a conventional HPLC. Another advantage of such systems is that they can be easily automated, providing high precisions and lower run times. On the other hand, if high sensitivity is not as important as easier handling and lower price, then appropriate is CE. When CE equipment is coupled to ED or fluorescence detector, it provides the lowest LODs possible, at a low nM range. Here there are no mobile phases, but buffers like borate and phosphate which are commonly applied. GC is the least used method for catecholamines' identification, more examined in the past century, where it met high acceptance. It is also less costly and simpler than HPLC and is a nice alternative for someone who wants a comfortable operation. A huge drawback of GC is the unavoidable derivatization to make these substances less polar and more volatile, which increases the overall run time. Great temperatures and destructive detections that exist in a GC are other disadvantages. GC-MS is the optimal apparatus, giving high sensitivities at low nM levels. The mobile phase here is called carrier gas and helium is the dominant one. Even so, argon could provide even smaller LOQ values than helium and is appropriate as well.

Crucial is for one to know what apparatus should be used if the sample is taken from a specific biological material or when a specific catecholamine or metabolite is to be detected. When NE, E, and DA only should be determined either from urine, plasma or brain, HPLC-ED with isocratic elution seems the most suitable system, followed by CE-ED or CE-fluorescence. In some situations, where certain diseases or pathological states should be diagnosed, an isolated compound that acts as a biomarker must be quantified.

For example, analysts tried to detect DA apart from NE and E in various articles. Mainly brain samples were taken and HPLC-ED with isocratic elution was much applied for this purpose. Isolated metanephrines could be observed at low LOD values with HPLC-MS/MS from human plasma, using gradient conditions. Finally, when micellar compounds and metabolites are to be simultaneously determined, various apparatuses and conditions can accomplish this, like HPLC-ED from brain samples with isocratic elution, CE-UV from urine samples, and GC-MS from human urine or plasma, with helium as a carrier gas, with increasing sensitivity values, in the order mentioned. This rumination is not settled, as hundreds of trials occur every day, and many different and special conditions are proven that may provide the anticipated goal. The above assumptions are broad considerations about the identification and determination of catecholamines and related compounds, especially in the last decades, and through this DT, one can observe some interesting and unusual schemes applied.

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