Automated Determination of VMA, HVA, 5-HIAA in Urine Using the Gilson GX-274 and Gilson ASPEC™ XL

Application Note CL0111

Keywords

Gilson GX-274, TRILUTION® LH, Catecholamines, Urine, Vanillylmandelic acid (VMA), Homovanilic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), Tumors, Diagnosis, Biosynthesis, Recipe

Introduction

The information included within this application note was provided by Martin Knirsch from Recipe, Chemicals + Instruments GmbH, Munich, Germany; www.recipe.de.

The catecholamines epinephrine, norepinephrine and dopamine fulfill a number of vital functions within the central and periphere nervous system. The analysis of catecholamines and their metabolites is of importance for the diagnosis of tumors of the sympathoadrenal system [1, 2], e.g. pheochromocytoma, neuroblastoma, and ganglioneuroblastoma [3-7]. Those tumors are responsible for an elevated catecholamine biosynthesis and secretion. As a result, significantly enhanced concentrations of catecholamines and metabolites are found in plasma and urine.

Sample cleanup and preparation are essential prior to the analysis of biological samples in order to remove proteins and other interfering compounds prior to analysis. Recipe has utilized both the Gilson ASPEC™XL (ClinRep® HPLC complete kit, see figure 1) and Gilson GX-274 with 402 Dual Syringe Pumps (see figure 2) to automate the sample cleanup and preparation process.

Beyond, a number of diseases are known to be accompanied by an increased or decreased activity of the catecholamine metabolism [8-12]. Figure 3 shows synthesis and metabolism of the catecholamines. Thus, VMA is a metabolite of epinephrine and norepinephrine, HVA a metabolite of dopamine.

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Serotonin, another biogenic amine, is mainly located in the enterochromaffine cells of the small intestine. Biochemically, serotonin is degraded by the enzymes monoaminooxidase (MAO) and aldehydedehydrogenase (Ald-DH) to 5-hydroxyindoleacetic acid (5-HIAA), see figure 4. Malignant growth in the enterochromaffine cells of the intestine results in increased production of serotonin and hence an increased excretion of 5-HIAA.

Figure 1. Recipe ClinRep® HPLC Kit Consumables

Figure 2. Gilson GX-274 with 402 Dual Syringe Modules

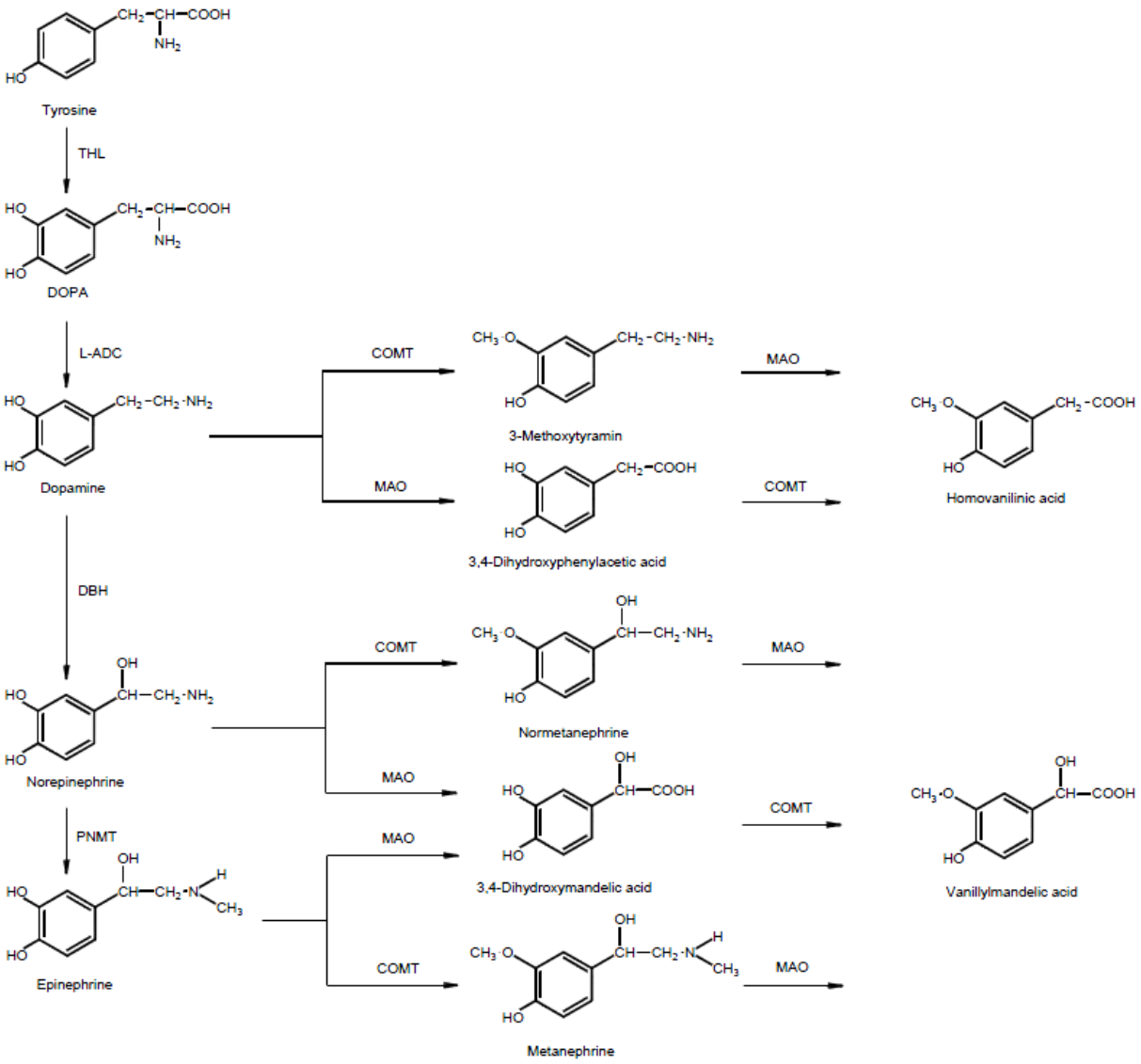


Figure 3. Biosynthesis and Metabolism of the Catecholamines

Abbreviations:

COMT: catechol-O-methyltransferase
DBH: dopamine-β-hydroxylase
THL: tyrosinehydroxylase,
DOPA: 3,4-dihydroxyphenylalanine
L-ADC: L-amino acid decarboxylase
MAO: monoamino oxidase
PNMT: phenylethanolamine-N-methyltransferase

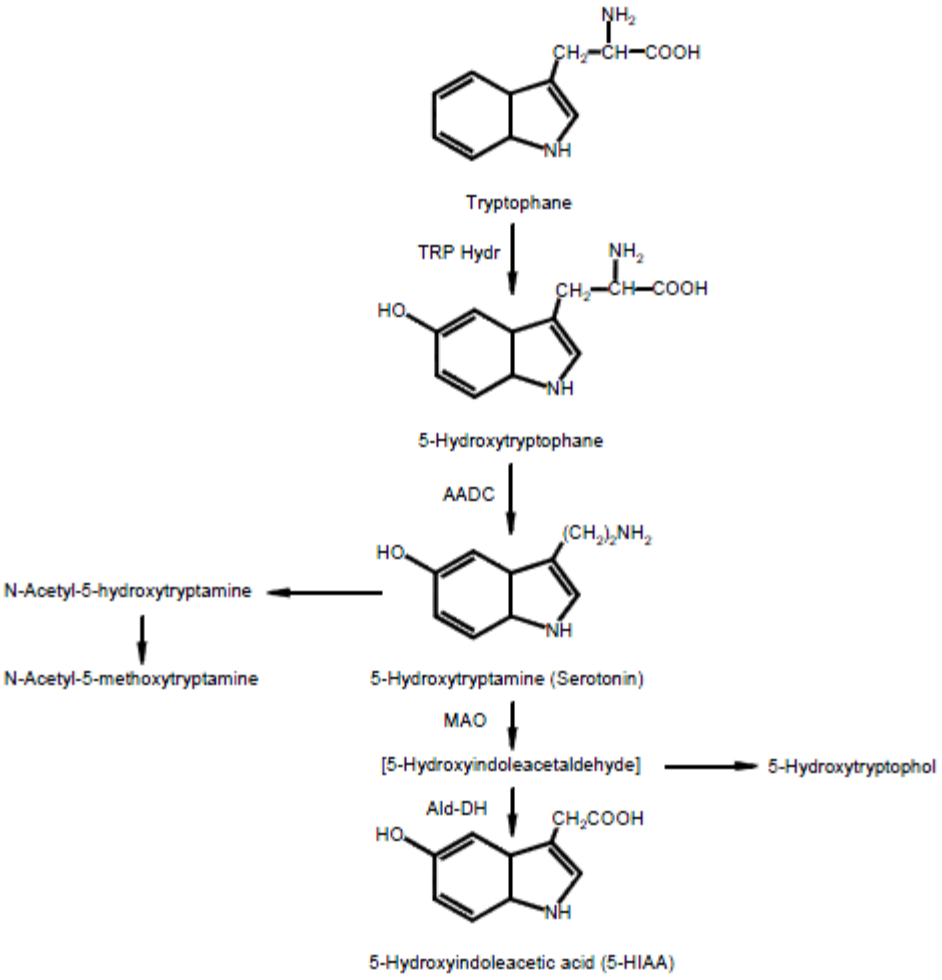


Figure 4. Metabolismus of Tryptophane

Abbreviations:

TRP Hydr: tryptophane hydroxylase
AADC: aromatic-L-amino acid decarboxylase
MAO: monoamine oxidase
Ald DH: aldehydedehydrogenase

For the determination of catecholamines and metabolites high performance liquid chromatography (HPLC) is considered as reference method, due to the fact that cross reactions may occur between parent substance and metabolites with the currently available immunoassys [e.g. see [12]).

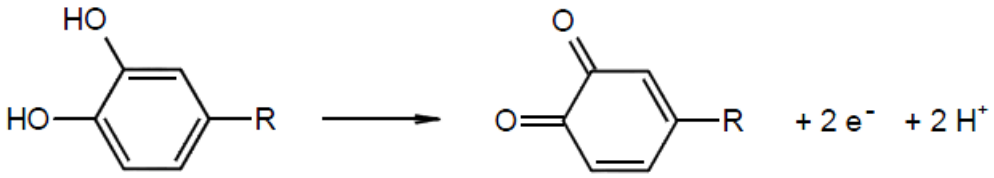
- ClinRep® HPLC complete kits for the determination of VMA, HVA and 5-HIAA in urine automated sample processing (order nos. 3100 - 3200)

Using the Recipe test kit, the analytes VMA, HVA and 5-HIAA are determined by electrochemical detection (ECD), a method of high sensitivity, specificity and selectivity. The specificity and selectivity of ECD is a result of detecting only oxidized or reduced compounds at the surface of a working electrode. The electrochemical turnover of a substance at the working electrode leads to a loss or to an uptake of electrons, resulting from oxidation or reduction. The arising current is proportional to the concentration of the substance flowing through the detector cell. The current is detected by a measuring device, amplified and displayed as a chromatogram.

Molecules which may be easily oxidized always have certain functional groups, in particular:

- hydroxyl groups on the benzene ring
- amino groups on the benzene ring
- thiol groups
- heterocyclic N- and S-atoms

Thus, the detection of e.g. catecholamines may be represented as follows:



In general, sample preparation has to be performed prior to the injection of samples onto the analytical system. Sample preparation serves to provide both sample clean-up and conversion of the analytes into a detectable form, respectively. Sample preparation is performed fully automated by the Gilson ASPEC™ XL or the Gilson GX-274. After sample injection, a special reversed-phase column is used for the HPLC separation of the analytes. The analytes are measured by EC detection and, using the internal standard method, are quantitatively evaluated via peak areas subsequently to calibration.

Materials & Methods

HPLC Conditions:

Mobile Phase Flow Rate: 0.9 mL/min.
Injection Volume: 20 µl
Run Time: 15 minutes
Gilson System Washing Solution: 10 % Methanol : 90% Water
Analytical Column: Heated at 30 °C
Detector: RECIPE ClinLab® EC Detector, Model EC3000
Retention times: VMA: ~ 3.7 minutes
Internal Standard: ~ 5.5 minutes
HVA: ~ 7.9 minutes
5-HIAA: ~ 11.6 minutes

GX-274 with two 402 Dual Syringe Modules
Automated Sample Preparation

The samples are automatically processed by the GX-274 with two 402 Dual Syringe Modules or the ASPEC™ XL. An overview of the automated SPE sample preparation with the GX-274 with two 402 Dual Syringe Modules is provided below:

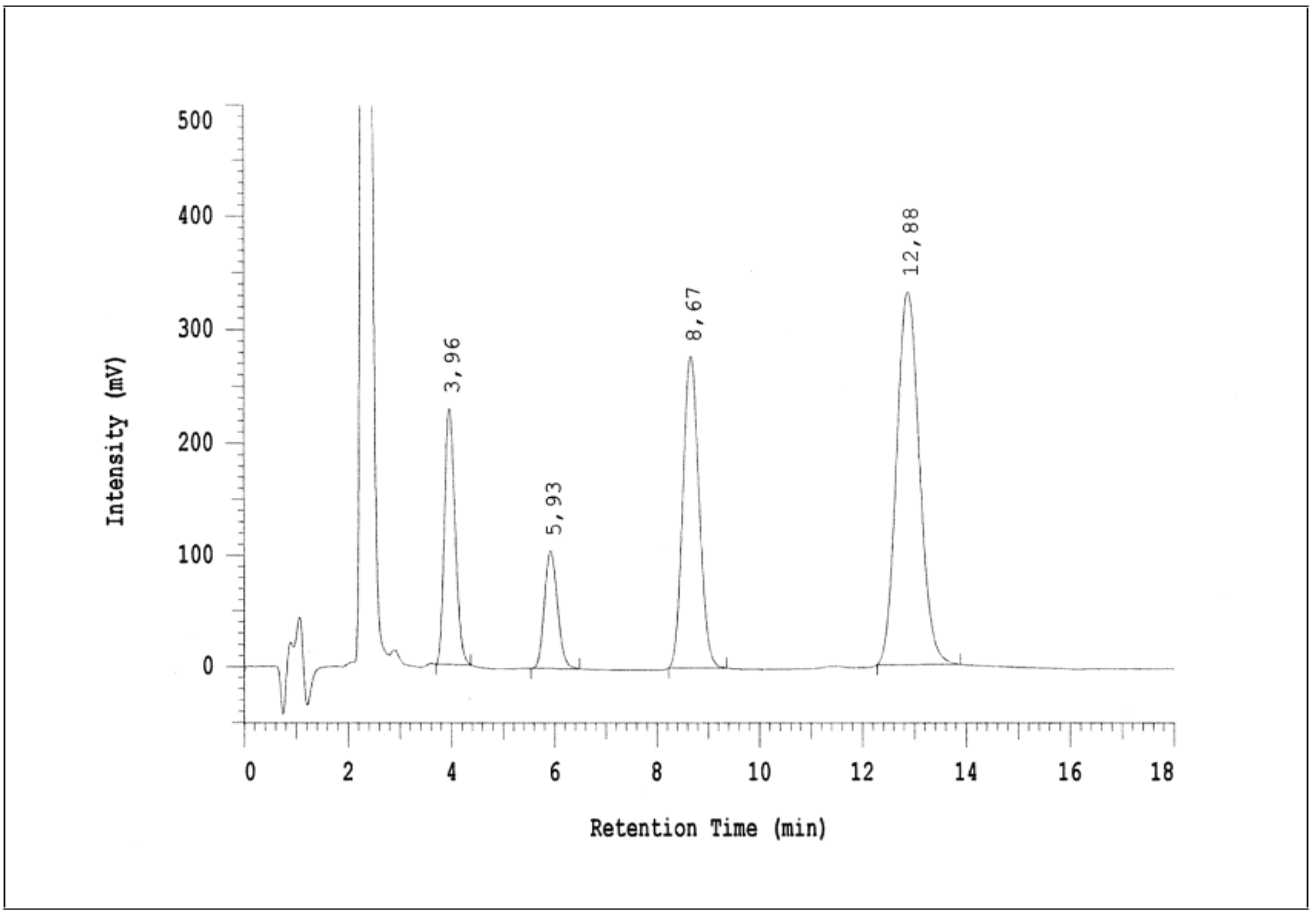
1. Dispense 50 µL urine sample and 1 mL internal standard (IS) to vial.
2. Mix dispensed urine and IS.
3. Load 1 mL mixed sample to SPE column.
4. Wash with 1 mL Ammonia Solution A.
5. Wash with 2.5 mL Boric Acid B.
6. Wash with 2.5 mL Boric Acid B.
7. Elute 2 mL Eluting Reagent E.

Note: Mix the eluted fraction from the Gilson GX-274 ASPEC. Using the HPLC system, inject 20 µL eluted fraction after mixing.

Figure 5. TRILUTION® LH Solid Phase Extraction Method Using the GX-274 System

Results

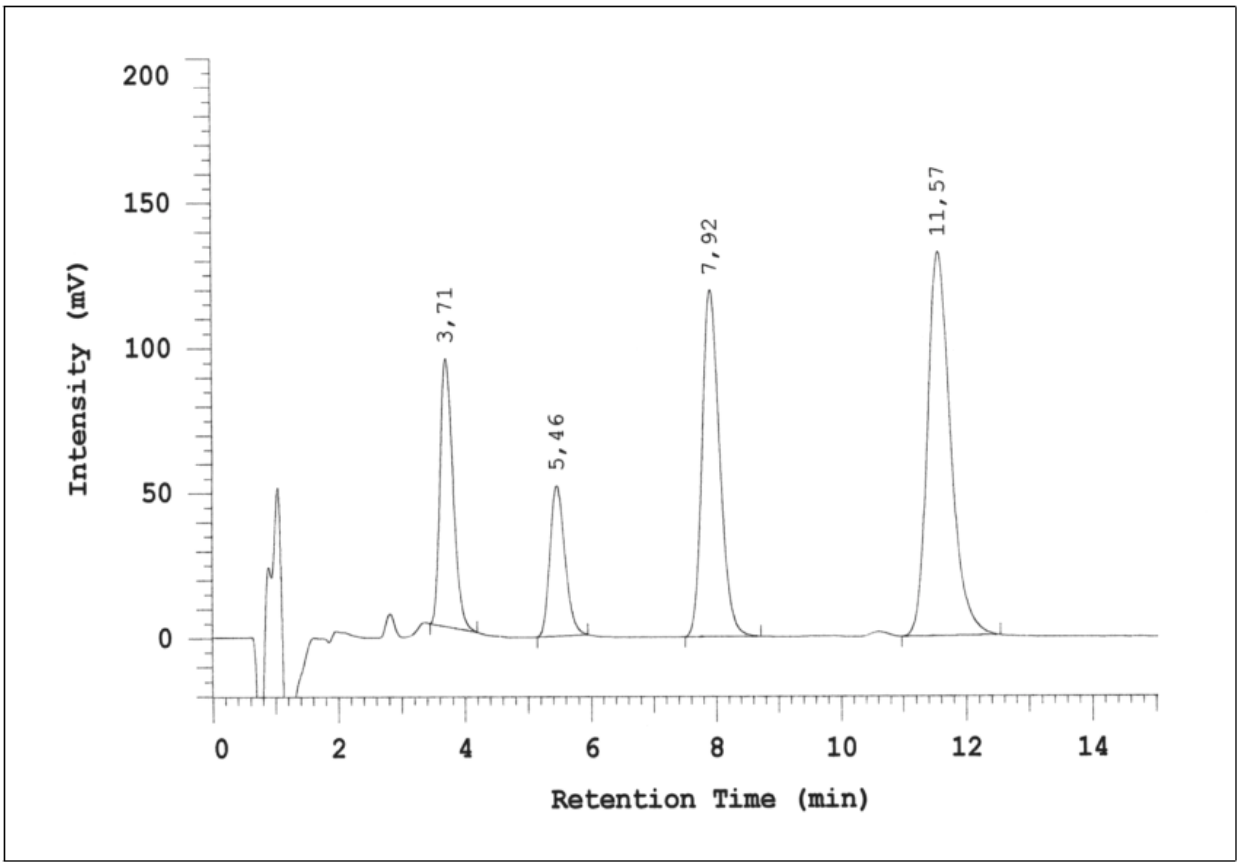
Figure 6. Example Chromatogram of Standard Solution (Recipe Order No.: 3011)



Retention Times:

VMA: 3.96 minutes
Internal Standard: 5.93 minutes
HVA: 8.67 minutes
5-HIAA: 12.88 minutes

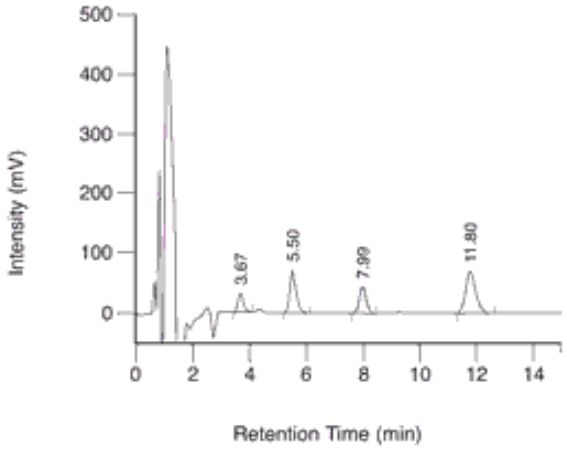
Figure 7. Example Chromatogram of Prepared Urine Calibrator (Recipe Order No.: 3013)



Retention Times:

VMA: 3.71 minutes
Internal Standard: 5.46 minutes
HVA: 7.92 minutes
5-HIAA: 11.57 minutes

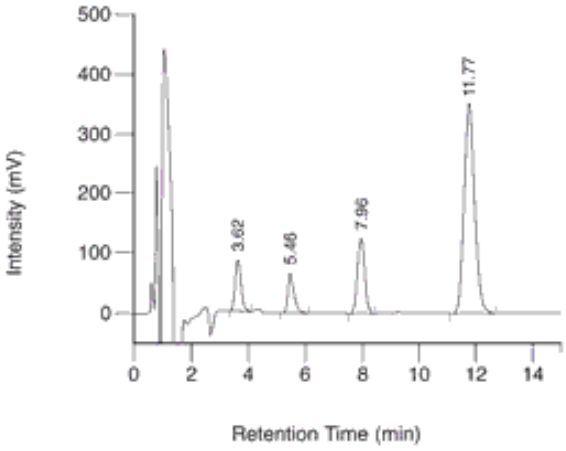
Figure 8. Example Chromatogram of ClinChek® Urine Control, Level I



Retention Times:

VMA: 3.67 minutes
Internal Standard: 5.50 minutes
HVA: 7.99 minutes
5-HIAA: 11.80 minutes

Figure 9. Example Chromatogram of ClinChek® Urine Control, Level II



Retention Times:

VMA: 3.62 minutes
Internal Standard: 5.46 minutes
HVA: 7.96 minutes
5-HIAA: 11.77 minutes

Recovery:

For evaluation of recovery native blank urine was spiked with VMA, HVA, 5-HIAA and the Internal Standard. Each sample was prepared 4-fold and subsequently was determined by two different HPLC systems. Related to a standard solution, that was injected directly onto the HPLC system, mean recovery rates from 75 - 89% were found.

Table 1: Intraassay Precision

SP = Sample Preparation

Gilson ASPEC™ XL was used to generate intraassay precision results

Sample		VMA CV [%]		HVA CV [%]		5-HIAA CV [%]	
		SP ASPEC™	SP manual	SP ASPEC™	SP manual	SP ASPEC™	SP manual
Sample 1	Range	0,87-2,23	1,39-2,85	0,90-2,82	1,53-2,99	0,11-5,27	1,83-4,48
	Mean value	1,46	2,03	1,69	2,24	2,13	2,66
Sample 2	Range	0,84-1,99	2,10-3,97	1,23-2,64	1,86-2,99	1,22-5,76	1,72-4,13
	Mean value	1,31	3,14	2,10	3,03	2,91	2,79
Sample 3	Range	0,62-1,95	1,15-3,88	0,82-2,85	1,42-4,48	0,95-4,02	1,83-4,00
	Mean value	1,28	2,37	1,92	2,62	2,46	2,26

Table 2: Interassay Precision

SP = Sample Preparation

Gilson ASPEC™ XL was used to generate intraassay precision results

Sample	VMA CV [%]		HVA CV [%]		5-HIAA CV [%]	
	SP ASPEC™	SP manual	SP ASPEC™	SP manual	SP ASPEC™	SP manual
Sample 1	2,24	4,31	3,35	4,17	3,30	3,97
Sample 2	1,12	2,85	1,42	3,06	2,00	1,92
Sample 3	1,96	4,03	2,98	4,07	2,63	4,86

Summary

Analyzing catecholamines and their metabolites is important for tumor diagnosis in both urine and plasma samples. This application note discussed the automated sample preparation and solid phase extraction extraction for patient urine samples performed with the Gilson ASPEC™ XL or Gilson GX-274 with 402 Dual Syringe Pumps. The subsequent SPE fractions are analyzed via HPLC for analytes VMA, HVA, and 5-HIAA using a highly sensitive, specific, and selective electrochemical detection (ECD) method.

Automating the sample preparation process and solid phase extraction extraction process increases laboratory efficiency and reduces laboratory error. Using the internal standard, recovery from integrated analytical runs can be calculated to verify the overall SPE process is consistent and within expected limits. Typical recovery rates for this assay range from 75 – 89%.

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