

o-CRESOL and PHENOL IN URINE by FLUORIMETRY - FAST –
Code Z55315

BIOCHEMISTRY

Phenol is used in many organic syntheses of industrial interest and as a disinfectant. It is toxic by itself and is absorbed through the skin, respiratory system, and in accidental cases, through the digestive system. In the organism phenol is partially conjugated with sulfuric acid and glucuronic acid. There is no bioaccumulation and is excreted in the urine.¹

TOXICITY

Acute poisoning: by accidental ingestion occurs and leads to convulsions and rapid collapse. Phenol is irritating to mucous membranes, eyes and skin. Chronic intoxication leads to esophageal pain, headaches, dizziness, anorexia, and acidosis, nausea, vomiting and diarrhea.¹

The classification of the carcinogenicity of phenol is as follows:²

EPA-I;D, IARC-3, MAK-3B, TLV-A4

This method allows to determine the concentration of phenol in urine after hydrolysis.

o-Cresol is a urinary metabolite of toluene, a substance that is used in the production of everyday materials in current use (glues, paints, lacquers, inks, etc..) and is also present as an environmental micro pollutant. This means that the entire population is exposed to small concentrations of Toluene. Additionally, an important source of Toluene indoors is tobacco smoke.³

Toluene is absorbed through the lungs and more slowly through the skin. In organisms it is initially found in the blood and subsequently deposited in adipose tissue. 20% of the absorbed dose is eliminated through the respiratory system, 80% is biotransformed: the major metabolites are hippuric acid, o-cresol and benzoic acid.¹

TOXICITY

Acute exposure: irritation of the upper respiratory tract and depression of the central nervous system. Chronic Exposure: alcohol intolerance, headache, and sleep-wake rhythm disorders, symptoms in the liver (hepatomegaly) and the kidneys (nephrotoxicity).¹

The urinary excretion of o-cresol is very low: the rate of elimination is approximately 1.08 mg / h and there is notable interindividual variability. Average concentrations of 38 mg / g creatinine were found in males and 25 mg / g creatinine in females.³

The dose in urine is an indication of exposure to Toluene.

The classification of the carcinogenicity of o-Cresol is as follows:²

EPA-C, MAK-3A, TLV-A4

This method can be used to determine the concentration of o-cresol in the urine after hydrolysis.

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This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 004	o-Cresol and Phenol in urine by Fluorimetry - FAST	11/2025
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TECHNICAL FEATURES

Principle of the Method :

o-Cresol and Phenol, after hydrolysis at 95 ° C for 30 minutes, are first buffered and then diluted with appropriate solvents; the solution is directly injected into HPLC.

Recovery : >98 %

Analytical Sensitivity (LLOD) : 0,013 mg/l o-Cresol
0,01 mg/l Phenol

Minimum concentration analyzed (LLOQ) : 0,04 mg/l o-Cresol
0,03 mg/l Phenol

Linearity : o-Cresol 0,04 – 10 mg/l
Phenol 0,03 – 500 mg/l

Accuracy intra serie (relative error %)
o-Cresol:

Ci	Cs
0,1 mg/l	0,6 mg/l
6,4%	3,9%

Accuracy inter serie (relative error %)
o-Cresol :

Ci	Cs
0,1 mg/l	0,6 mg/l
7,4%	5,2%

Reproducibility intra serie (coefficient of variation %) o-Cresol:

C LLOQ	Cm	Cs
0,04 mg/l	0,3 mg/l	1 mg/l
8,7%	3,6%	2,9%

Reproducibility inter serie (coefficient of variation %) o-Cresol:

C LLOQ	Cm	Cs
0,04 mg/l	0,3 mg/l	1 mg/l
10,8%	6,4%	3,4%

Coefficient of correlation R² + Dev Std 0,9958 ± 0,0015 o-Cresol

Accuratezza intra serie (errore relativo %)
Phenol :

Ci	Cs
10 mg/l	30 mg/l
3,8%	1,0%

Accuratezza inter serie (errore relativo %)
Phenol :

Ci	Cs
10 mg/l	30 mg/l
6,4%	3,5%

Riproducibilità intra serie (coefficiente di variazione %) Phenol :

C LLOQ	Cm	Cs
0,03 mg/l	20 mg/l	50 mg/l
8,5%	5,9%	3,4%

Riproducibilità inter serie (coefficiente di variazione %) Phenol :

C LLOQ	Cm	Cs
0,03 mg/l	20 mg/l	50 mg/l
9,8%	6,6%	4,4%

Coefficiente di correlazione R² + Dev Std 0,9955 ± 0,0020 Phenol

Biological Exposure index – B.E.I.:

o-Cresol in urine: 0,3 mg/g creatinine
Phenol in urine: 250 mg/g creatinine
ACGIH 2020

Components of the kit (500 tests) :

All reagents are ready to use and stable 3 years at Room Temperature ($\leq 20^{\circ}\text{C}$), except **Reagent C** for which reference should be made to see the Warnings.

Reagent B – Buffer Solution, **1 x 450 ml**

Reagent C – Test Solution, **1 x 2 ml**

See Warnings

Reagent D – Internal Standard Solution, **1 x 5 ml**

Urine Calibrator, **5 x 1 ml**

Code Z55016 (packed separately – see data sheet)

Reagent M – Mobile Phase, **15 x 500 ml**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 5 μl
Fluorimetric Detector $\lambda_{\text{EX}}=270\text{ nm}$ $\lambda_{\text{EM}}=300\text{ nm}$
Chromatograms Recorder

Optional Equipment:

Autosampler.
Operational Computer

Urine Collect Procedure :¹

For Phenol:
Sampling time: end of turn.
Container: polyethylene or polypropylene tubes
Conservant: not necessary
Storage: at 4°C till 7 days

For o-Cresol:
Sampling time: end of the shift (ACGIH), after a prolonged period of exposure at the end of exposure or shift (DFG)
Container: polyethylene tubes
Conservant: not necessary
Storage: at 4°C till 7 days

PREANALYTICAL PROCEDURE

Preparation of Test Solution.

Pipette in a tube :

- 980 µl of H₂O HPLC grade
- 10 µl of **Reagent C – Test Sol.**
- 10 µl of **Reagent D – Internal Standard Sol.**

Vortex for 10 sec.

INJECTION :

- Inject 5 µl of this solution in the chromatographic system.

Verify that the Test Solution has retention time similar to fig. 1. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

Important : Don't use this solution to calibrate!

ANALYTICAL PROCEDURE

STEP 1 : Samples preparation

Dispense in pyrex tubes with teflon caps:

	Calibrator	Sample
Calibrator	100 µl	
Sample		100 µl
HCl37%(concentrated hydrochloric acid)	150 µl	150 µl

Vortex for 10 sec.

STEP 2 :Incubate at 95 °C for 30 minutes

Cooling at Room Temperature

STEP 3 : Add 850 µl of **Reagent B – Buffer Solution** + 10 µl of **Reagent D – Internal Standard Sol.**

Vortex for 10 sec.

N.B.: at this step the sample is stable 1 day at 2-8 °C

Note: For the determination of phenol, in the presence of a peak in saturation on the L2 control, it is possible to further dilute the buffered solution or even better, decrease the injection volume

INJECTION :

- Inject 1 - 5 µl of this solution in the chromatographic system.

o-CRESOL and PHENOL IN URINE - Warnings

REAGENT C : TEST SOLUTION

o-CRESOL	15,0 mg/l
PHENOL	20,0 mg/l
Store at 2-8 °C	

FLUORIMETRIC DETECTOR PARAMETERS

λ_{EX}	270 nm
λ_{EM}	300 nm
SENSITIVITY	Medium

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL 120 PFP (4,6 x 50 mm, 2,7 μ m), the use of Javelin Prefilters (1 x 10 pcs) Code S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical Reversed-Phase column POROSHELL 120 PFP (4,6 x 50 mm, 2,7 μ m). Disconnect the detector and filter 30 ml of a solution of H₂O : Acetonitrile (20:80 v/v) set flow at 1,4 ml/min discharge first 28 ml. **Don't recycle the washing solutions.** Condition the column with the mobile phase for 10 minutes. Condition further on the column for 10 minutes also at recycling phase. **It is NOT possible to make analysis at recycling phase.** If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

CLEANING AND STORAGE COLUMN

Disconnect the detector. Flux a solution of H₂O : Acetonitrile (20 : 80 v/v) at flow of 1,4 ml/min for 20 min and discharge it. Flux 10 ml of H₂O : Acetonitrile (80 : 20 v/v) for 10 min at flow of 1,0 ml/min and discharge it.
The column should be stored tightly closed in a solution of H₂O : Acetonitrile (20 : 80 v/v)

WASH NEEDLE INJECTION

The injection needle should be washed with a solution of H₂O : Methanol or Acetonitrile or Ethanol (80 : 20 v/v)

HPLC PARAMETERS

LOOP	5 μ l
FLOW	1,4 ml/min.
PRESSURE	About 150 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z55016	Calibrator for o-Cresol and Phenol in urine	4 x 1 ml
Z55019	Control for o-Cresol and Phenol in urine - Levels 1 and 2	2 x 5 x 1 ml
Z55311	Mobile Phase	4 x 500 ml
Z699975408	Poroshell 120 PFP (50 x 4,6mm –2,7 μ) Analytical Column	1 Pc
S90199511	Javelin Prefilters	1 x 10 Pcs
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs

BIBLIOGRAPHY:

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- 8- Brega, Grandini, Amaglio, Pafumi. Determination of phenol, m-, o- and p-cresol, p-aminophenol and p-nitrophenol in urine by HPLC. J Chromatogr 1990 Dec 28;535 (1-2):311-6.
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- 10- Nakashima, Kinoshita, Wada, Kuroda, Baeyens. HPLC with fluorescence detection of urinary phenol, cresols and xylenols using 4-(4,5-diphenyl-1H-imidazol-2-yl) benzoyl chloride as a fluorescence labelling reagent. Analyst. 1998 Nov; 123(11):2281-4.

o-CRESOL and PHENOL IN URINE (Reference Chromatograms)

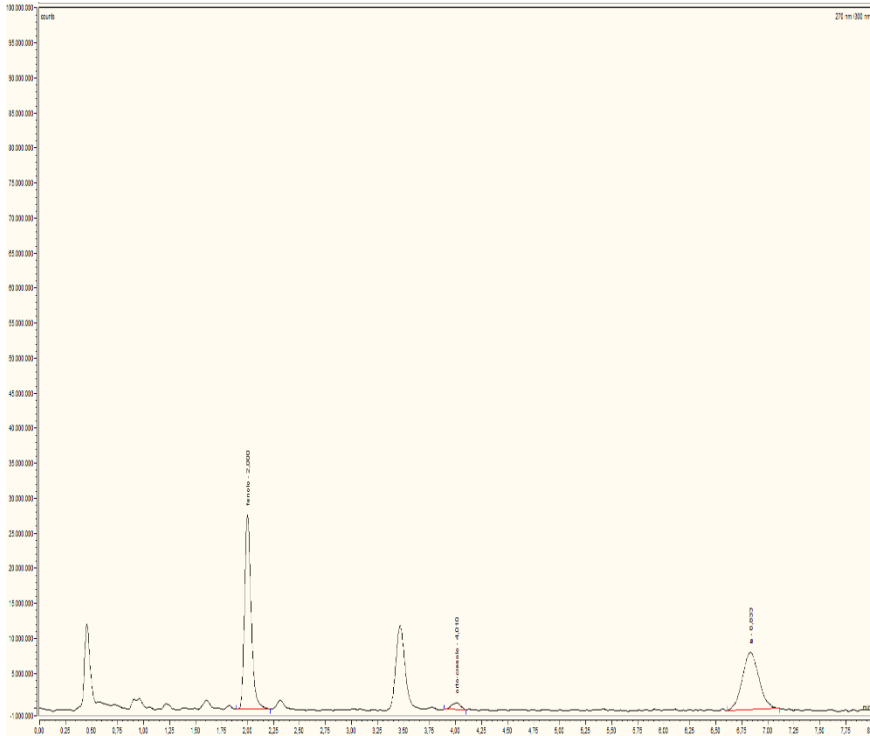


Fig. 1	Urine Calibrator
	R.T. 2.0 Phenol 7,6 mg/l
	R.T. 4.0 o-Cresol 0,5 mg/l
	R.T. 6.8 Internal Standard

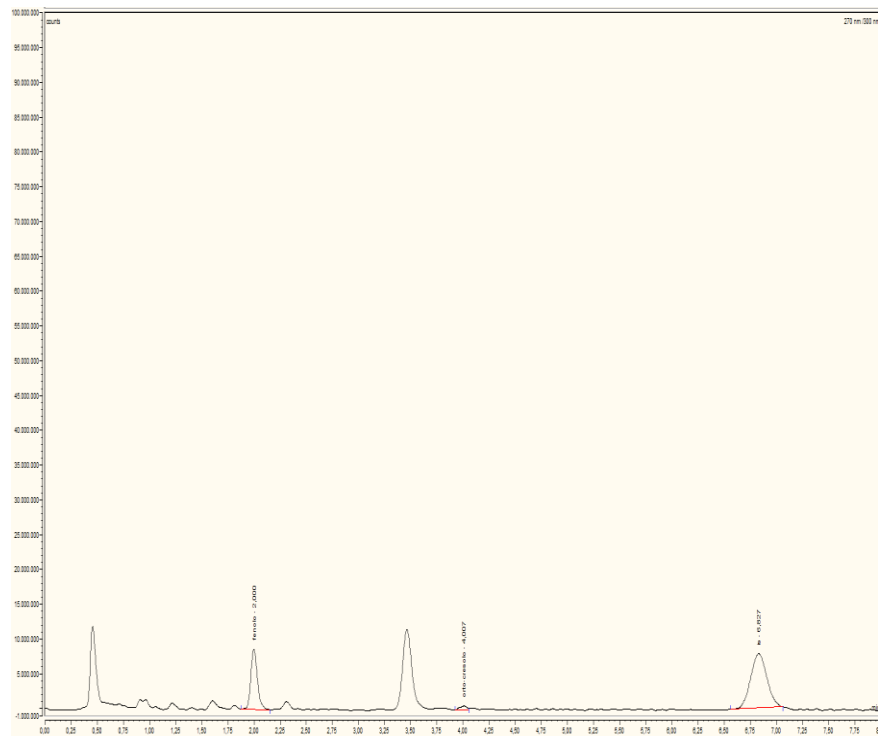


Fig. 2	Urine Control Level 1
	R.T. 2.0 Phenol 2,5 mg/l
	R.T. 4.0 o-Cresol 0,18 mg/l
	R.T. 6.8 Internal Standard

o-CRESOL and PHENOL IN URINE (Reference Chromatograms)

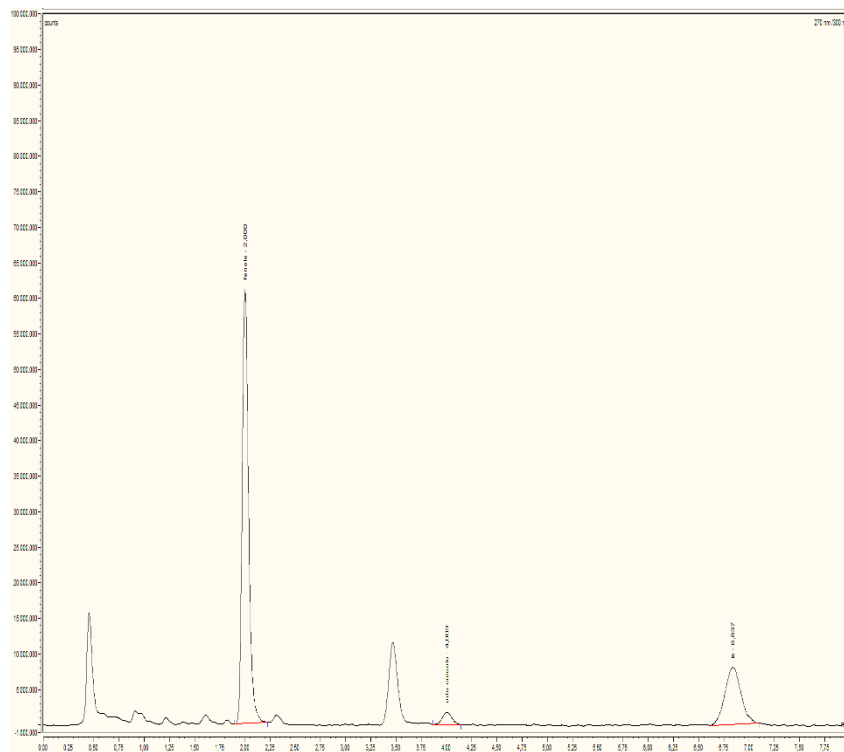


Fig. 3	Urine Control Level 2
	R.T. 2.0 Phenol 16,4 mg/l
	R.T. 4.0 o-Cresol 1,0 mg/l
	R.T. 6.8 Internal Standard