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METHOD FOR MASS CONCENTRATION DETERMINATION OF PHENOL AND PYROCATECHOL IN BLOOD USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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The relevance of determining phenol and its main metabolite pyrocatech in the biological environment is related to the professional activities of workers of organic synthesis, bisphenols, dyes, varnishes, synthetic resins, plasticizers, stabilizers, pesticides and other industrial and household products. working conditions are characterized by the effect of phenol and pyrocatechin on health status.

Phenol is an intermediate product of the metabolism of proteins and aromatic amino acids in the human body. As a natural product of metabolism, phenol is formed in the blood in small amounts and is excreted in the form of conjugate in the urine.

Pyrocatechin is the main metabolite of phenol. Exogenous entry of phenol into the human body occurs through inhalation of contaminated air and skin contact.

It is necessary to develop and improve methodological support for determining the mass concentration of phenol and pyrocatechin in the blood at background values and higher levels to assess the risk of production - environmental factors affecting the health of workers. It is a highly sensitive and selective method of high performance liquid chromatography.

Several methods are known for determining the amount of phenol in blood using gas chromatography:

(Method 1).There is a known method for determining free phenol in blood using gas chromatography with a flame ionization detector. Detection is based on preliminary conversion of phenol into methylphenyl ether by interaction with methyl iodide in

an alkaline medium, isolation of the derivatization product from blood by extraction with diethyl ether, and subsequent gas chromatographic analysis of the extract. The calibration characteristic is determined by the internal standard (naphthalene) method in phenol calibration mixtures based on whole blood. Xylenes and o-, m-, p-cresols do not interfere with detection. The sensitivity of determining the mass concentration of phenol in blood is 0.04 mg/dm^3, the error does not exceed 25%.

There is a known method for determining free phenol and o, m, p - cresol in blood serum by gas chromatography with detection in a flame ionization detector. According to the method, the extraction of phenols and cresols from blood serum was carried out by steam distillation followed by extraction with chloroform. Separation of analytes was carried out on a glass packed column (2 m x 4 mm) filled with Chromaton-N-AW-DMCS (0.20-0.25) mm sorbent containing dinonyl phthalate (10%) and the stationary liquid phase of Chromaton-N increased. It is coated with -AW-DMCS (0.16-0.20) mm OV-17 (phenyl (50%) methyl silicone oil). Highly pure nitrogen was used as the carrier gas at a flow rate of 50 ml/min. Column temperature 140 ^{0}C, evaporator temperature 180 ^{0}C. Sample analysis was performed in isothermal mode. The volume of the injected sample is 2 to 5 µl. Phenol identification was done by relative retention time. Quantification of phenol was performed using an internal standard method with o-cresol. Detection sensitivity is 2.0 mg/dm^3.

Preparation of calibration solution

Name of solvents	1	2	3	4	5	6
The volume of the working solution, 5 mg/dm^3, cm^3	0,02	0,05	0,25	0,5	2,5	5
Protein concentration in the calibration solution, mg/dm^3	0,004	0,01	0,05	0,1	0,5	1
Concentration of phenol in the calibration solution, mg/dm^3	0,004	0,01	0,05	0,1	0,5	1

Results of the analysis of the concentration of phenol and pyrocatechol in the blood (working samples)

No	Phenol in blood, mg/dm^3		Pyrocatechin in blood, mg/dm3	
	Parallel detection result	The result of parallel detection using the arithmetic mean method	Parallel detection result	The result of parallel detection using the arithmetic mean method
1	0,0041 0,0043	0,0041	0,0070 0,0062	0,0066
2	0,0141 0,0159	0,0150	0,0180 0,0160	0,0170
3	0,0061 0,0055	0,0058	0,0201 0,0179	0,0190
4	0,0045 0,0040	0,0043	0,0053 0,0047	0,0050
5	0,0147 0,0147	0,0140	0,1339 0,1262	0,1301
6	0,0042 0,0038	0,004	0,0312 0,0288	0,0300
7	0,0027 0,0030	<0,004	0,0432 0,0408	0,0420
8	0,0042 0,0037	0,004	0,0198 0,0222	0,0210
9	0,0042 0,0038	0,004	0,0093 0,0103	0,0098
10	0,0003 0,0003	<0,004	0,0065 0,0057	0,0061

A method for the determination of the mass concentration of phenol and pyrocatechol in blood by high-performance liquid chromatography, taking a blood sample, acidifying it with a 0.1% solution of orthophosphoric acid in a 20:1 volume sample/acid solution ratio, and extracting analytes by liquid extraction using acetonitrile to acidify the sample the sample/acetonitrile volume ratio of 1:1 is centrifuged at 5000 rpm for 10 minutes, the upper layer of the extract is selected, and 0.1 g of the mixture of C18 sorbent and magnesium sulfate salt in a 1:6 mass ratio is added to it, respectively; mixed for 1 minute to purify the extract and centrifuged for another 10 minutes at 5000 rpm, the purified extract was filtered and 10 mm^3 of the filtered extract was introduced into the chromatograph, measured and determined using the phenol concentration and pyrocatechol calibration graph.

References

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