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Development and validation of a portable gas chromatograph method for quantitative determination of oxygen and toxic gas impurities in medical oxygen using planar microchromatographic columns and detectors

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Abstract: This study examines portable Gas Chromatography (GC) for the quantitative analysis of oxygen and impurities, focusing on the development and validation of a method to determine oxygen, carbon monoxide, carbon dioxide, methane, and nitrogen in medical compressed oxygen gas. The goal is to ensure the quality of medical-grade oxygen. The method's validation assessed its metrological characteristics, demonstrating specificity through clear chromatographic separation of the target gases and the absence of these peaks in the carrier gas chromatogram. It exhibited linearity within the designated concentration ranges, while precision met permissible standards, with the relative standard deviation for intermediate precision being less than 4 % for carbon monoxide (0.00025 - 0.00099 %), less than 3 % for methane (0.0005 - 0.00246 %) and carbon dioxide (0.0050 - 0.0150 %), less than 2 % for nitrogen (0.1 - 0.7 %), and less than 0.01% for oxygen (99.27 - 99.98 %). Overall, the validation results confirm the suitability of this analytical method for the quantitative determination of the aforementioned gases in medical compressed oxygen using portable GC with microchromatographic columns and detectors.

Key words: oxygen, medical gases, gas chromatography, planar microchromatographic columns, quality control

1. Introduction

Oxygen is an indispensable life-saving medicine, crucial for maintenance therapy in treating respiratory diseases such as COVID-19 and pneumonia. Pneumonia alone causes around 800,000 deaths annually, with estimates suggesting that 20 - 40 % of these could be prevented through oxygen therapy.¹ Additionally, it is vital for surgical procedures, injury treatments, and resuscitation efforts. Vulnerable populations,

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including the elderly, pregnant women, and newborns, often require regular oxygen therapy.^{1,2} The COVID-19 pandemic has significantly increased the global demand for oxygen, highlighting the urgency of its supply. In low- and middle-income countries, demand has surged to 1.1 million cylinders, while in the Russian Federation, medical oxygen demand has risen by over 40 %.³⁻⁵

Oxygen production is complex, involving both specialized medical equipment and industrial enterprises. It is widely used across various industries, including healthcare. However, medical-grade oxygen differs significantly in purity and quality from industrial oxygen, which is not suitable for human use. Medical oxygen must be tested for authenticity, purity, and quantitative content according to an approved pharmacopoeial monograph. Proper methods for production, storage, and distribution are essential to ensure patient safety. Uncertainties about the purity of industrial oxygen, potential particulate matter, microbial contamination, and improper handling can pose unacceptable risks to patients.⁵

Quality control of medical oxygen is a very important task. Oxygen is supplied to medical institutions mainly in reusable containers and cylinders, both in gaseous and liquid form. Since the containers are used for filling repeatedly, there is a high probability of oxygen quality deterioration in a particular vessel used in a hospital, and not in the entire batch of oxygen. In this regard, technologies are needed that allow the analysis of oxygen supplied to the patient directly in a medical facility, without transporting it to the laboratory. Moreover, transporting an oxygen cylinder or liquid oxygen sample to the laboratory is a difficult task, since oxygen is classified as dangerous goods, and the relevant testing laboratory can be located several hundred kilometers from a medical facility.

Depending on the source and production method, medical oxygen may have the different percentages. As per the requirements of the International Pharmacopoeia, European Pharmacopoeia, Russian Pharmacopoeia, etc, oxygen for medical use, obtained by the method of liquefaction (cryogenic distillation) of air, must contain at least 99.5 %.⁶⁻⁹ Oxygen concentrators used as one of the sources of oxygen must provide a continuous supply of pure and concentrated oxygen at a low flow rate with a content of at least 82 %.³ In addition to the requirements for the quantitative oxygen content for medical oxygen with its specification of 99.5 % and 93 %, there are also requirements for the content of impurities, such as carbon monoxide (less than 0,0005 %), carbon dioxide (less than 0,0100 %), water (less than 0,0067 %).⁷⁻⁹

Various methods can be used to control the quality of medical oxygen. For the quantitative determination of oxygen, the most common in the world is the use of a paramagnetic analyzer.⁶⁻⁸ Chemical absorption methods of analysis are also used.⁶⁻⁸ To control the content of impurities, IR analyzers or qualitative reactions are used.⁶⁻⁸ The use of gas analyzers based on the paramagnetic effect of oxygen or IR absorption allows one to accurately determine the analyzed components, however, their use requires several devices (sensors) for each component to be determined separately, and does not allow one to detect the presence of other possible undeclared components in oxygen as well as unspecified impurities.⁶⁻⁸ The accuracy of the test when using the same chemical methods of analysis is highly dependent on the experience of the operator and the quality of reagents and utensils.

In this paper, for the quantitative determination of oxygen, carbon monoxide, carbon dioxide, methane, and nitrogen in medical compressed oxygen gas, a gas chromatography method is proposed, which is characterized by versatility and high sensitivity, using a portable chromatograph based on planar technologies and microfluidic systems. To confirm the reliability and reproducibility of the developed methodology, its validation was carried out. Validation of analytical methods proves the validity of the choice of method and conditions for the analysis, increases the degree of quality assurance of medicines. Validation of the analytical method was carried out according to the characteristics such as specificity, accuracy, linear response, the detection limit, the quantitation limit and, precision. The possibility of using the gas chromatography method for the quantitative determination of oxygen, carbon monoxide, carbon dioxide, methane, and nitrogen in medical compressed oxygen gas by the gas chromatography method was also evaluated and comparative tests were carried out with the methods given in the current Russian Pharmacopeia monograph 2.2.0026.18 "Medical oxygen gas" in terms of the content of carbon monoxide and carbon dioxide and quantitative determination.⁶

According to European Pharmacopeia monograph 11.2. 01/2010:0417 'Oxygen', the content of carbon monoxide and carbon dioxide in medical compressed oxygen gas should be less than 0.0005 % and 0.01 %, respectively.⁷ The assay of oxygen should be at least 99.5 %. During method development using gas chromatography, impurities not controlled in the current pharmacopoeia monograph were detected in medical compressed gas samples. These impurities, such as methane (ranging from 0.0014 to 0.0020%) and nitrogen, are related to the oxygen production process. During method validation, these impurities were also included in the validation process. The developed method was successfully applied during the COVID-19 pandemic as part of the state quality control of medicines in mobile laboratories of the Federal Service for Surveillance in Healthcare of Russia (Fig. 1 of supplementary information) and is still used today. The developed method formed the basis for the changes made to the pharmacopoeia monograph of the Russian Pharmacopoeia 2.2.0026.20 "Oxygen, medical compressed gas". Over 800 oxygen samples have been

analyzed using this method. The samples included oxygen from the hospital supply system, liquid oxygen, and oxygen in cylinders. Tests were conducted both routinely and in emergency situations. This was done during a period of acute oxygen shortage to ensure delivery to patients in need.

2. Experimental Section

For the development and validation of the method, the portable gas chromatograph "PIA" (LLC "NPF MEMS", Samara, Russian Federation) was used with a thermal conductivity micro detector (TCD), a thermochemical micro detector (DTC) and the following planar columns (*Fig.* 1): micro packed planar gas chromatographic column 2 m long, 1 mm in diameter with Carboxen 1000 sorbent, micro packed planar gas chromatographic column 2 m long, 1 mm in diameter with Porapak N (DVB-EVBEthyleneglycol-dimethacrylate) sorbent, micro packed planar gas chromatographic column 2 m long, 1 mm in diameter with NaX molecular sieve sorbent.¹⁰⁻¹⁵

The chromatograph has 3 independent chromatographic lines. Chromatographic separation of the sample occurs along all three lines simultaneously, which can significantly reduce the analysis time. The analyzed or calibration mixture enters the channels of the microdosers. To switch gas flows, each microdoser is equipped with two electrically controlled pneumatic distributors, which are mounted on a flat plate.



Fig. 1. Optical image of as-developed planar microfluidic chromatographic column. The process of creating the planar gas chromatography columns consists of the following steps: the first stage (*Fig.* 1(a)): channels formed on the aluminum plate by milling (0.6×0.6 mm - cross-section; 0.8 m length); the second stage (*Fig.* 1(b)), the resulting aluminum plate with channels is connected with another one by the chemically inert thermoplastic resin



Fig. 2. Comparison of chromatograms of reference gas mixture batch No. 8565 (1) and carrier gas (helium grade A) (2) (enlarged view). (a) separation of oxygen, carbon monoxide and methane; (b) separation of oxygen and carbon dioxide; (c) separation of oxygen and nitrogen. The concentration of the determined substances is presented in *Table* 1.

Table 1. Composition of reference gas mixtures

Component	Component volume	Expanded	Component volume	Expanded		
	fraction, %	uncertainty*	fraction, %	uncertainty*		
	№929	00	Nº798	№ 7980		
N_2	0.4970	0.0070	0.1027	0.0026		
CO	0.000251	0.000010	0.00485	0.00012		
CH4	0.00146	0.00004	0.00491	0.00012		
CO_2	0.00494	0.00012	0.01023	0.00026		
O_2	99.496s3		99.8773			
Component	Component volume	Expanded	Component volume	Expanded		
	fraction, %	uncertainty*	fraction, %	uncertainty*		
	№ 8565		№6223			
N_2	0.7060	0.0110	0.0102	0.00026		
CO	0.00099	0.00004	0.000552	0.000022		
CH4	0.00246	0.00006	0.000429	0.000017		
CO_2	0.0150	0.0004	0.001067	0.000027		
O_2	99.2756		99.9878			

*At coverage factor k = 2. Corresponds to the limits of absolute error with confidence probability P = 0.95.

Pneumatic distributors have two operating positions "electric control on" (set position) and "electric control off". In this case, analytes or calibration mixtures fill the doses of columns for chromatographic separation, as a result of which the detectors record chromatograms of the analyzed components.¹⁵

Due to the use of planar and microfluidic technologies, a small sample volume is consumed for analysis, only 250 μ l, and the consumption of carrier gases and electricity is also low. For carrier gases, small-capacity cylinders with a volume of 0.7 - 1 L are used, and a portable battery is enough to power the chromatograph. The miniaturization of the device made it possible to place all its components in a protective

shockproof case, allowing its use in the field (see *Fig.* 2 of supplementary information).

Helium for chromatography and dry compressed air were used as carrier gases. The work used standard gas mixtures (Monitoring LLC, Russian Federation), the composition of which is given in *Table* 1.

For each of the three chromatographic lines used, the optimal chromatographic conditions were selected for the determination of the corresponding analytes. Chromatographic columns were designed to determine carbon monoxide and methane (*Table* 2(a), the volume ratio of carbon dioxide (*Table* 2(b), and the volume ration of oxygen and nitrogen (*Table* 2(c)).

Table 2. Chromatographic compound for carbon

Table 2. (a) To determine carbon monoxide and methane, we design the following chromatographic column

Parameters	Specification
Column Type	Micropacked chromatographic column
Column Diameter	1 mm
Column Length	2 m
Column Filling Material	Carbon molecular sieves (Carboxen 1000)
Grain Size	60/80 mesh
Column Thermostat Temperature Mode	Isothermal
Operating Temperature	70-80 °C
Carrier Gas Type	Compressed air class 0
Carrier Gas Flow Rate	10 ± 2 mL/min
Detector Type	Thermal conductivity detector
Sample Volume	250 μl
Analysis Time	4 min

(b) To determine the volume ratio of carbon dioxide, we design the following chromatographic column

Parameters	Specification
Column Type	Micropacked chromatographic column
Column Diameter	1 mm
Column Length	2 m
Column Filling Material	Porapak N
Grain Size	80/100 mesh
Column Thermostat Temperature Mode	Isothermal
Operating Temperature	70 °C
Carrier Gas Type	Helium (Grade A)
Carrier Gas Flow Rate	10 ± 2 mL/min
Detector Type	Thermal conductivity detector
Sample Volume	250 μL
Analysis Time	4 min

(c) To determine the volume ration of Oxygen and Nitrogen, we design the following chromatographic column

Parameters	Specification
Column Type	Micropacked chromatography column
Column Diameter	1 mm
Column Length	2 m
Column Filling Material	80/100 mesh molecular sieves (NaX)
Column Thermostat Temperature Mode	Isothermal
Operating Temperature	$40 - 60 {}^{\mathrm{o}}\mathrm{C}$
Carrier Gas Type	Helium (Grade A)
Carrier Gas Flow Rate	10 ± 2 mL/min
Detector Type	Thermal conductivity detector
Sample Volume	250 μL
Analysis Time	4 min

monoxide and methane determination, the volume ratio of carbon dioxide, and the volume ration of oxygen and nitrogen,

Into the dosing device of the chromatograph, which

is a dosing valve for gas samples, 250 μ l of the test gas and a standard sample were injected at a pressure of about 20(±1) kPa. At least three chromatograms were obtained for each sample. The retention time

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on the first gas chromatographic line of peaks of carbon monoxide is about 63 seconds, methane is about 156 seconds, on the second line of carbon dioxide is about 77 seconds, on the third line of oxygen is about 77 seconds, nitrogen is about 120 seconds.

The content of impurities in the sample in percent (X) was calculated by the formula:

$$X_1, \% = \frac{X_0 \times S_1}{S_0}$$

Where S_1 and S_0 are average values of the peak areas of carbon dioxide on the chromatograms of the test and standard samples, respectively and X_0 – concentration of sample gas in the reference gas mixture.

The oxygen content (X_{O2}) was calculated by the formula:

$$X_{O_2}, \% = 100 - \sum X_i$$

Where X_i the content of each of the impurities in volume ratios in the tested medicinal product. Reference gas mixture was used as standard and tested samples.

3. Results and Discussion

To ensure the applicability of the developed method, it was validated according to the following parameters: specificity, accuracy, linear response, the detection limit, the quantitation limit, precision. The specificity of the method was carried out to determine the influence of the mobile phase on the peaks of impurities and the main substance. When testing the technique by the "Specificity" parameter, the chromatogram of the carrier gas shows the absence of peaks belonging to carbon monoxide and carbon dioxide, methane,

Table 3. Method uncertainty calculation

nitrogen and oxygen (*Figs.* 2 and 3 of Supplementary Information) and *Fig.* 4 (Supplementary Information)).

Fig. 2 shows chromatograms for determining microimpurities that may be present in medical oxygen. The analysis time for methane and carbon monoxide (*Fig.* 2(a)) is 160 sec, for carbon dioxide (*Fig.* 2(b)) it is 100 seconds, and the retention time for nitrogen is 140 sec. Therefore, one analysis cycle to determine all the required compounds takes 180 seconds, significantly reducing labor costs for analyzing medical oxygen.

3.1. Resolution

When checking the specificity, the resolution of closely eluting peaks in the chromatograms of the calibration gas mixtures was calculated, which should be at least 1.5. The resolution between the peaks of oxygen and carbon monoxide was 2.2, between the peaks of oxygen and carbon dioxide was 5.0, between the peaks of oxygen and nitrogen was 2.5.

3.2. Accuracy

To determine the accuracy, three calibration gas mixtures were used with the content of carbon monoxide in the concentrations of 0.00025%, 0.00055%, 0.00099%, methane in the concentrations of 0.0005%, 0.00146%, 0.00246%, dioxide carbon in the concentrations 0.005%, 0.010%, 0.015%, nitrogen in the concentrations of 0.1%, 0.5%, 0.7%, oxygen in the concentrations of 99.9%, 99.5%, 99.3%. Each sample was chromatographed at least three times. When defining reproducibility for quantitative determination, the relative standard deviation was calculated, which usually should not exceed 2% for the main component (oxygen) and 10% for impurities. The

Component	SRD, %	Max SRD for Reference gas mixture, %	Corresponding peak area SRD, %	Standard uncertainty (relative), %	Extended uncertainty (relative), %
N_2	1.14	0.025	2	2.3	4.5
CO	3.30	0.04	2	3.9	7.6
CH_4	2.40	0.04	2	3.1	6.1
CO_2	2.91	0.255	2	3.5	6.9
O_2	0.006	0.36	2	2.0	3.9

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Carbon monoxide concentration, %	Carbon monoxide peak area	Carbon monoxide peak height	Methane concentration, %	Methane peak area	Methane peak height
0.000251	1.166	0.300	0.000429	3.028	0.346
0.000552	3.181	0.696	0.00146	13.244	1.512
0.00099	5.536	1.438	0.00246	24.453	2.705
0.00485	30.71	7.007	0.00491	50.867	5.668
Carbon dioxide concentration, %	Carbon dioxide peak area	Carbon dioxide peak height	Nitrogen concentration, %	Nitrogen peak area	Nitrogen peak height
0.001067	Below detection limit	Below detection limit	0.0102	Below detection limit	Below detection limit
0.00494	1.572	0.317	0.1027	5.7388	3.7388
0.01023	3.761	0.729	0.497	132.972	15.198
0.015	5.806	1.119	0.706	198.737	23.132
Oxygen concentration, %		Oxygen Peak Area		Oxygen p	eak height
99.2756		33331.8		305	5.916
99.4963		33782.5		305	8.503
99.8773		34518.8		3057.798	
99.987	/8	34790		305	9.456

Table 4. Relation between the areas of the peaks of the determined components and their concentrations

obtained metrological characteristics are shown in *Tables* 1-5 (Supplementary Information). Also, for each determined component, the uncertainty was calculated (*Table* 3).

3.3. Response

To demonstrate the linear response of the method, four samples presented in *Table* 4 were measured. Based on the data obtained, linear calibration relationships between the analyte concentration and the response were created.

Based on the data obtained, plots of the relation of the peak area on the concentration of carbon monoxide, methane, carbon dioxide, and nitrogen were done. The value of the square of the correlation coefficient must be at least 0.99. According to *Fig.* 3, the square of the correlation coefficient for all determined components was ≥ 0.999 . Therefore, the dependence of the peak area on the concentration is linear. Similarly, the dependence of the peak height on concentration is also linear for all determined impurities. The height of the oxygen peak in the given concentration range remains constant.

The detection limit (DL) was calculated from the standard deviation of the area and the slope of the



Fig. 3. The peak areas of impurities and oxygen relate to the concentration of components. This dependence is based on the analysis of test gas mixtures, with component concentrations shown in *Table* 3.

plot of the dependence of the peak height on the concentration according to the formula:

$$DL = 3, 3 \cdot \sigma/S,$$

Where σ – the standard deviation of the response,

S – the slope of the calibration curve.

Thus, the detection limit for carbon monoxide is 0.0001 %, methane 0.0005 %, carbon dioxide 0.0033 %, nitrogen 0.0214 % (*Table* 5). The detection limit for oxygen is significantly below the minimum allowable content in medical compressed oxygen and is not calculated in this work.

The quantitation limit (QL) was set by the value of the standard deviation of the signal and the slope of

Table 5. DL and QL values for the method

Component	DL	QL
N ₂	0.0214%	0.065%
CO	0.0001%	0.0004%
CH_4	0.0005%	0.0016%,
CO_2	0.0033%	0.0100%
O_2	-	-

the graph according to the formula:

$$QL = 10 \cdot \sigma/S$$

Where σ – the standard deviation of the response, S – the slope of the calibration curve.

Thus, the quantitation limit of determination for carbon monoxide is 0.0004 %, methane 0.0016 %, carbon dioxide 0.0100 %, nitrogen 0.065 % (*Table 5*). The quantitation limit of oxygen is significantly lower than the minimum allowable content in oxygen in medical compressed gas and is not calculated in this work.

Additionally, *Fig.* 5 (Supplementary Information) shows a fragment of the "noise" of the baseline on the chromatogram of a helium solution. The value of the baseline "noise" signal height was 0.06. Therefore, the minimum value of the signal height of impurities, at

Table 6. The content of impurities and oxygen obtained during the analysis of a reference gas mixture

Sample	Carbon monoxide content, %	Methane content, %	Carbon dioxide content, %	Nitrogen content, %	Oxygen content, %
Sample 1	0.00022	0.00145	0.00462	0.5256	99.4681
Sample 2	0.00026	0.00145	0.00555	0.5164	99.4763
Sample 3	0.00021	0.00143	0.00516	0.5202	99.4730
Sample 4	0.00025	0.00149	0.00510	0.5012	99.4920
Sample 5	0.00025	0.00144	0.00473	0.4869	99.5067
Sample 6	0.00026	0.00144	0.00511	0.4872	99.5060
Mean value, %	0.00024	0.00145	0.00505	0.5063	99.487
SD, %	0.000021	0.000021	0.00033	0.0169	0.017
Confidence interval $(P = 0.95), \%$	±0.000022	±0.000022	±0.00035	±0.0178	±0.0178

Table 7. The content of impurities and oxygen in the analysis of samples of reference gas mixture

Sampla	Content of carbon monoxide, %		Methane of	Methane content, %	
Sample	1st set factor	2nd set factor	1st set factor	2nd set factor	
Sample 1	0.00022	0.00022	0.00148	0.00148	
Sample 2	0.00023	0.00024	0.00139	0.00134	
Sample 3	0.00025	0.00022	0.00150	0.00130	
Sample 4	0.00024	0.00023	0.00147	0.00135	
Sample 5	0.00022	0.00024	0.00143	0.00139	
Sample 6	0.00023	0.00025	0.00147	0.00133	
Mean value, %	0.00023	0.00023	0.00146	0.00136	
SD, %	0.000017	0.000011	0.000038	0.000065	
Confidence interval $(P = 0.95), \%$	± 0.00004	± 0.00003	± 0.00010	±0.00016	

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Table	7.	Continued
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Comula	Content of car	bon dioxide, %	Nitrogen content, %		
Sample	1st set factor	2nd set factor	1st set factor	2nd set factor	
Sample 1	0.00468	0.00455	0.4880	0.4838	
Sample 2	0.00475	0.00561	0.4830	0.4813	
Sample 3	0.00470	0.00528	0.4815	0.4718	
Sample 4	0.00483	0.00584	0.4834	0.4747	
Sample 5	0.00563	0.00538	0.4848	0.4625	
Sample 6	0.00540	0.00549	0.4840	0.4635	
Mean value, %	0.0050	0.00530	0.4841	0.4729	
SD, %	0.00044	0.00044	0.0022	0.0088	
Confidence interval $(P = 0.95), \%$	±0.0011	± 0.0011	± 0.0057	±0.0226	
Samula		Oxygen content, %			
Sample		1st set of factors	2nd set of factors		
Sample 1		99.5057	99.5099		
Sample 2		99.5107	99.5115		
Sample 3		99.5121 99.5214		99.5214	
Sample 4	Sample 4		99.5101 99.5178		
Sample 5		99.5079	99.5305		
Sample 6		99.5089	99.5294		
Mean value, %)	99.5092	99.5201		
SD, %		0.0023	0.0087		
Confidence interval (P = 0.95), %		± 0.0024	± 0.0092		

which they can be reliably determined, is 0.6. The intermediate accuracy of the method was verified in the course of a multivariate experiment (during two working days by two different analysts on two instruments) on the same reference gas mixture. According to *Tables* 6 and 7, the results of quantitative determination proved the satisfactory precision and stability of the developed method.

4. Conclusions

During the research, it was found that the method of gas chromatography using a portable gas chromatograph and planar microfluidic columns allows obtaining reliable results, and can also be used for the quantitative determination of carbon monoxide and carbon dioxide, methane, nitrogen, oxygen in medical compressed oxygen gas.

Thus, it was shown that for the analysis of oxygen according to the parameters established in the mono-

graph of the Russian Pharmacopoeia 2.2.0026.18 "Medical oxygen gas", it is possible to use a chromatograph based on planar chromatographic columns and microfluidic systems, which can be used not only in stationary laboratory conditions, but also directly at the objects of production, storage or consumption of oxygen, including in emergency situations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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