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UV-SPECTROMETRIC AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF PENTOXIFYLLINE IN WORKPLACE AIR

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ABSTRACT

Pentoxifylline is a pharmaceutical drug used to eliminate or to reduce disturbances in the peripheral blood circulation. Two methods with the same sampling and sample preparation for determination of pentoxifylline in workplace air are developed. Air sampling at workplace is made by means of perchlorovinyl filters (FPP). A twice methanol extraction for 20 minutes from FPP-filters is used. A simple UV-spectrometric procedure is described. The average recovery is 85.3 – 95.03 %. The method is not selective. A selective reversed-phase liquid chromatographic method for the determination of pentoxifylline in the air of working environment is developed, too. Aliquot (50 µl) of extract is injected and then separated on a reversed phase RP LiChrosorb C_{18} Perkin Elmer column (5 µm, 250 mm x 4.6 mm i.d). The mobile phase is acetate buffer (pH 4.3): acetonitrile 3:7 (v/v) with flow rate 1.0 cm^3 min^{-1}. The UV-detection at 275 nm is used. The average recovery is 86.8 – 94.1 %.

Keywords: *pentoxifylline, spectrometry, reversed phase liquid chromatography, workplace air, UV detection.*

INTRODUCTION

Pentoxifylline (Fig. 1) is a synthetic three substituted xanthine derivative, applied to the treatment of cerebral and cerebrovascular diseases. The pentoxifylline has a similar structure like another xanthine derivatives – caffeine, theophilline and theobromine.

Pentoxifylline is white or almost white crystalline powder, soluble in water ($\approx$77 mg ml^{-1} at 25°C), freely soluble in methylene chloride, sparingly soluble in alcohol ($\approx$63 mg ml^{-1} at 22°C) and very slightly soluble in ether [1].

During the drug production (recipe preparation, granulation, encapsulation and packing) the contamination of the workplace air with the pentoxufylline aerosols is possible. It is know that in the air of workplace pentoxifylline may be found like disintegrated aerosol [2]. Workers are exposed to pentoxifylline principally by inhalation, where the aerosols are rapidly absorbed

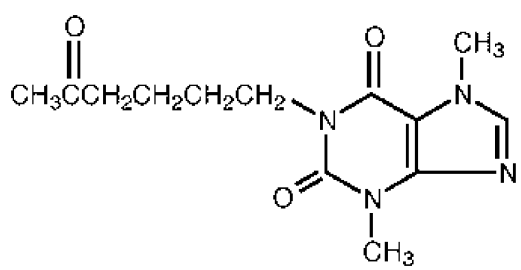

Fig. 1. Chemical structure of pentoxiphylline.

into the body. Exposure by skin absorption or ingestion may also occur. In literature causes of enhanced sensibility by processing xanthines are described. Causes of headache, insomnia, and lost of memory are described from workers after occupational exposure by xanthine derivatives [3].

Because the chemical factors are of particular significance for the occupational risk assessment, methods for determination of pentoxifylline concentrations in workplace air have been developed.

Health legislation sets no limit value for pentoxifylline [5], but there is a time-weighted average (TWA) for caffeine of 1.0 mg m^{-3}, theophilline – 0.5 mg m^{-3} and theobromine 0.5 mg m^{-3} [4].

Gaschromatographic methods [6] and a high-performance liquid chromatographic procedure [7,8] have been described for determining pentoxifylline in biological samples and pharmaceutical forms only. These methods are oriented to determinate the bioavailability of pentoxifylline in milk or blood and to examine the pharmaceutical substance.

The methods reported in this paper are suitable for sampling from air and sufficiently reliable for the determination of pentoxifylline in the working environment.

EXPERIMENTAL

Reagents and chemicals

All chemicals used were gradient grade. Methanol, acetonitrile, glacial acetic acid and ammonium acetate were purchased from Merck. The pentoxifylline, caffeine and theophilline standard substances were supplied by Uni-Pharm, Bulgaria. The stock solution was prepared by dissolving 100 mg pentoxifylline in 100 cm^3 methanol. Acetate buffer was prepared through adjusting of 0.02 mol dm^{-3} ammonium acetate solution to pH 4.3 with glacial acetic acid.

Sampling and sample preparation

Sampling. It is known that pentoxifylline can be found in the air at workplace as a disintegrated aerosol with an average size of particles about 2.5 μm and σ_g = 3.2. The air aspiration from workplace atmosphere at 2 to 20 $cm^3\ min^{-1}$ through the FPP-15 perchlorovinyl filters is the suitable method for sampling of pentoxifylline aerosols. It uses the pump, calibrated at working rate of air delivery with a relative accuracy 5 %. The precision of sampling time measurement is ± 0.1 minute. The air volume is estimated like air at a normal condition (20°C, 1013 hPa) by the equation:

$$V_0 = \frac{V_t \times 293 \times P}{(273 + t) \times 1013}$$

where:

V_o is the volume adjusted to normal conditions;

t is the sampling temperature, °C;

V_t is the volume at the conditions of the sampling;

P is the sampling pressure.

Sample preparation. The FPP were two times extracted with 5 ml methanol for 20 min at a room temperature. The methanol extracts were transferred to a volumetric flask of 10 cm^3.

UV-spectrometric procedure

UV-specrtometry was performed by using spectrophotometer Perkin-Elmer "Lambda-5". Series standard solutions of pentoxyfilline in methanol were made fresh daily by diluting the stock solution. The wavelength of maximal absorption (272 nm) was determined by scanning of pentoxifylline standard solutions (from 5 to100 μg cm^{-3}).

The alteration of absorption in the presence of theophylline and caffeine is presented in Table 1.

Performance data obtained by the UV-spectrometry are presented in Table 2.

Table 1. The ateration of absorption of pentoxifylline at 272 nm in the presence of theofilline or caffeine.

Impending substance	Increase of absorption of pentoxifylline (in %) at ratio pentoxifylline : impending substance		
Proportion	1 : 0.1	1 : 0.5	1 : 1
Caffeine	+11.4	+72.3	+99.9
Theofilline	+8.2	+57.7	+81.6

Table 2. Performance data of the spectrometric method for determination of pentoxifylline in air.

№	Standard concentrations (μg.cm^{-3})	Number of trials	Average absorbance	Standard Deviation, S.D.	Coefficient of Variation C.V., (%)
1.	5.0	10	0.1715	0.0093	5.42
2.	10.0	10	0.3402	0.0083	2.44
3.	30.0	10	1.0327	0.01266	1.23
4.	50.0	10	1.7476	0.02162	1.23
5.	70.0	10	2.3865	0.01647	2.34
6.	100.0	10	3.0160	0.00916	0.30

Table 3. Parameters of the HPLC method for determination of pentoxifylline in air.

№	Standard concentrations (μg.cm^{-3})	Number of trials	Average heights of peaks (at equally sensitivity) (mm)	Standard Deviation S.D.	Coefficient of Variation C.V. (%)
1.	0.5	10	6.9	0.6726	9.74
2.	2.0	10	27.8	1,0351	3.72
3.	10.0	10	147.9	1.4289	0.96
4.	20.0	10	287.7	3.8819	1.35
5.	30.0	10	441.0	10.3095	2.34
6.	40,0	10	561.7	5.7009	1.01
7.	50.0	10	713.9	11.1668	1.56

The calibration graph absorption/concentration of pentoxifylline was linear. It is described by the equation:

$$Y = 30.84 \cdot X + 0.087$$

where Y is absorption at concentration X and the correlation coefficient is $R^2 = 0{,}9897$. The detection limit is 0.005 mg cm^{-3} and the relative standard deviation is 5.4 %. This measurement is not selective, because theophilline and caffeine interfere the spectrometric determination of pentoxifylline.

HPLC chromatography

HPLC was performed with a Perkin-Elmer series 4 system equipped with a injector-Reodyne 7125 and a UV Perkin Elmer LC 75 detector. Compounds are separated on a 250 mm x 4.6 i.d., C_{18} RP LiChrosorb reversed-phase column, 5 μm particle. The mobile phase was 0.02 mol dm^{-3} ammonium acetate buffer, pH 4.3 – acetonitrile, 70:30 (v/v), at a flow rate of 1 cm^3 min^{-1}. The column temperature was 30°C. Detection was performed at wavelength of 272 nm. (The UV-spectrum of pentoxifylline was acquired with a Perkin Elmer Spectrophotometer Lambda-5.) The retention time of pentoxifylline was 4.88 min and the retention time of caffeine and theophilline were 2.8 and 3.3 min, respectively. The optimum conditions showed the good separation of pentoxifylline, caffeine and theophilline.

The data obtained by the HPLC method are listed in Table 3. The calibration plot for pentoxifylline was linear in the range of 0.5 to 50.0 μg cm^{-3} in the injection volume of 50 μl. This wide linear range enabled determination of a large number of concentration in workplace air. Linear regression analysis gave the calibration equation:

$$Y = 14.227 \cdot X + 2.4732$$

The correlation coefficient was $R^2 = 0.9994$.

The recovery of the methods for determination of pentoxifylline in air was obtained using gravimetri-

Table 4. Recovery of pentoxifylline by UV-spectrometry and HPLC methods.

Total number of injections	Amount (g) added gravimertically to the filters	Quantity, (g) determined by UV-spectrometry	Recovery (%)	Quantity, (g) determined by HPLC	Recovery (%)
10	0.0050	0.00488	97.6	0.00479	95.8
10	0.0050	0.00505	101.1	0.00497	99.4
10	0.0049	0.00476	97.1	0.00471	96.1
10	0.0054	0.00508	94.1	0.00509	94.3
10	0.0051	0.00467	91.6	0.00462	90.6
10	0.0054	0.00479	88.7	0.00477	88.3
10	0.0021	0.00174	82.9	0.00183	87.1
10	0.0022	0.00195	88.6	0.00200	90.9
10	0.0024	0.0018	75.0	0.00184	76.7
10	0.0022	0.00227	103.2	0.00232	100.1
10	0.0021	0.00190	90.5	0.00194	92.4
10	0.0023	0.00165	71.7	0.00169	73.5

cally loaded FPP filters. Air (300 dm^3) was aspirated through the loaded filters and they were extracted with methanol (2x5 cm^3). The methanol extract obtained were diluted to a suitable concentration and analyzed by UV-spectrometry and by HPLC. The results obtained are presented in Table 4. The recoveries obtained for 0.002 g and 0.005 g pentoxifylline per filter were 85.3±13.41 and 95.03±4.71 % by UV – spectrometric method; 86.8±10.04 and 94.1±4.27 % by HPLC method.

CONCLUSIONS

Results obtained by both methods are in good comparability. The HPLC method with UV detection is more selective, than UV spectrometric method. The detection limit of HPLC method (0.5 μg cm^{-3}) is 10 times lower than the detection limit of UV spectrometric method (5 μg cm^{-3}). The high selectivity of the HPLC method for pentoxifylline, and the obtained detection limit allow the detection of amounts higher than time-weighted average in workplace air in the presence of other xanthines. The UV spectrometric method is simple and not required expensive equipment and it permits to determine pentoxifylline when the other xanthines are not available in workplace air. The presented methods are simpler than those described in the literature. They are also sufficiently precise and suitable for application for the monitoring of the contamination of workplace with pentoxifylline aerosols during the drug production.

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