Journal of Applied Life Sciences International

15(4): 1-6, 2017; Article no.JALSI.38741
ISSN: 2394-1103

Urine-kinetics of Low Molecular Polyethylene Glycols Following an Oral Capsule Ingestion

K. Baum[1*], S. Einwächter[2], M. Bibl[2] and B. Huppertz[3]

[1]Institute of Physiology and Anatomy, German Sport University Cologne, Germany.
[2]Clinic for Psychiatry, Psychotherapy, Psychosomatic Medicine, Educational Institution of Ruhr University Bochum (Fliedner Hospital), Ratingen, Germany.
[3]Department of Toxicology and Drug Monitoring, MVZ Labor Dr. Quade & Kollegen, Cologne, Germany.

Authors' contributions

This work was carried out in collaboration between all authors. Authors BH and KB designed the study. Author KB performed the statistical analysis and wrote the first draft of the manuscript. Author MB wrote the protocol. Author SE managed the analyses of the study. Author KB managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2017/38741
Editor(s):
(1) Ali Mohamed Elshafei Ali, Professor, Department of Microbial Chemistry, Genetic Engineering & Biotechnology Building, National Research Centre, Egypt.
Reviewers:
(1) Sadaf Shafique, Quaid-E-Azam Medical College Bahawalpur, Pakistan.
(2) Yasir Merhmood, Rashid Latif College of Pharmacy, Pakistan.
(3) P. K. Lakshmi, G. Pulla Reddy College of Pharmacy, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/22784

Original Research Article

Received 11th November 2017
Accepted 15th January 2018
Published 18th January 2018

ABSTRACT

Purpose: Urine marking is an alternative approach to supervise sample collection in order to prevent urine manipulation in the field of drug usage and the use of doping substances. Polyethylene glycols (PEG) with low molecular masses are already established as marker substances but their urine kinetics after oral capsule intake is not yet clearly described.
Methods: 26 subjects (both male and female) participated in the study. After determination of baseline urine they ingested a capsule containing 300 mg PEG (17 subjects PEG8 + PEG10, 9 subjects PEG8 + PEG 12; PEG8 = 370 g/mol, PEG10 = 458 g/mol, PEG12 = 546 g/mol) with 100 ml of fluid. Thereafter, subjects were instructed to completely drain the bladder every 15 minutes for the first hour and every 30 minutes for the following two hours after capsule intake. Thereafter, they were allowed to urinate spontaneously but had to notice the time on a protocol for a further 21 hours. From each sample, the PEG concentrations were determined by means of liquid chromatography mass spectrometry.

**Corresponding author: E-mail: baum@professor-baum.de;*

Results: PEG could be clearly detected in all subjects after 60 minutes, regardless of drinking volume, alcohol consumption, or urine flow. At the latest 22 hours following intake, the marker re-attained baseline values in all participants.
Discussion: PEG capsules can be used as safe means for urine marking in a time interval of at least 60 minutes between oral intake and urine sampling is respected. Furthermore, using different marker combinations, this method can be applied day by day in routine urine drug testing without interference with previously taken markers.

Keywords: Urine; manipulation; marker; drugs; doping.

1. INTRODUCTION

In the field of the drug testing market, urine tests have been proposed and used by many institutions all over the world [1-6]. This well-established method has its weakness mainly due to potential sample exchange and adulteration. There are two approaches to impede urine sample falsification: visual control of the urination process and the use of a urine marker prior to urination. Regarding the former, aside from the hardly detectable deception with clean urine such as external urine released via life-like penises, direct observation may psychologically burden both client and controller [7].

Both restrictions can be excluded via urine markers such as polyethylene glycols (PEG) with low molecular masses which were introduced by Gauchel et al. [8]. PEG is considered as nontoxic and safe [9,10,11]. Their oral absorption decreases with molecular mass (M) [12], for M=600 g x mol^{-1} a bioavailability of about 60% was observed decreasing to 10% for M = 1000 g x mol^{-1} [12]. To ensure the detection of an orally ingested PEG, a time interval of 45 minutes between intake and urine sampling has been proposed [13]. However, in the studies of Huppertz et al. [13] subjects ingested the marker as aqueous solutions. An alternative dosage form are PEG-containing capsules, which were used in a study by Jones et al. [7]. In that study, urine was collected between 31 and 42 minutes following ingestion. In 9 out of 79 samples the marker was not found in urine. According to the manufacturer licensed for the US market [14], the marker should be verifiable in urine 30 minutes after oral administration but, unfortunately, to our best knowledge, we could not find any scientific literature supporting this statement by experimental data.

The aim of the present study was, therefore, to determine the kinetics of PEGs in urine following an oral ingestion of PEG-containing capsules.

2. METHODS

The present study followed all the relevant national regulations and the tenets of the Declaration of Helsinki and was approved by the ethical committee of the University of Duisburg-Essen, Germany.

2.1 Subjects

Subjects were recruited by local announcements. Those who agreed to participate in the study after detailed oral and written information gave their written informed consent. They received 150 € after completing the task. 26 subjects (12 women, 14 men; age 38.7 ± 7.4 years, height 176 ± 8.3 cm, weight 77 ± 17 kg, BMI 25,0 ± 4.0 kg x m^2) participated in the study.

2.2 Urine Sampling and Marker Detection

Subjects entered the laboratory without any preceding nutritional restriction and emptied the bladder. From this urine a 10 ml sample was taken for baseline analysis of PEG concentrations. PEGs of molecular weights between 370 and 546 g/mol were detected (PEG8 = 370 g/mol, PEG9 = 414 g/mol, PEG10 = 458 g/mol, PEG 11 = 502 g/mol, PEG12 = 546 g/mol). Thereafter, they ingested a capsule containing 300 mg PEG with 100 ml of fluid. 17 subjects received a PEG8+PEG10 and 9 subjects a PEG8+PEG12 combination. All subjects were instructed to completely drain the bladder every 15 minutes for the first hour and every 30 minutes for the following two hours after capsule intake. Thereafter, they were allowed to urinate spontaneously but had to notice the time on a protocol for a further 21 hours. At each urination, the volume was measured and 10 ml withdrawn into a monovette for subsequent analysis of PEG concentrations. Throughout the 24 hours subjects were allowed to eat and drink ad libitum but had to write down quantity and type of foods and beverages.

2.2.1 Liquid chromatograph mass spectometry

A six-point calibration in water was performed for quantification. The calibration points used were 1, 2.5, 5, 10, 25 and 50 µg/ml.

PEG concentrations were analyzed on a Shimadzu LCMS 8050 with a LC Nexera 2 from Shimadzu and computed by means of the LabSolutions software from Shimadzu (Duisburg, Germany). The PEGs used as standards were provided by CS-Chromatographie (Langerwehe, Germany) and the internal standard Dimethoxy-tetraethyleneglycol by Sigma-Aldrich (Steinheim, Germany), all other solvents and chemicals were of LCMS-grade purchased from Carl Roth (Karlsruhe, Germany).

The PEGs were separated on a Raptor™ biphenyl-column 2.7 µm, 100 * 2.1 mm from restek with a water/methanol gradient containing ammonia-formiat and formic acid.

Buffer A: 2 mM amminiumformiate, 0.1% formic acid in water.

Buffer B: 2 mM amminiumformiate, 0.1% formic acid in methanol.

The flow was 0.35 ml/min and started with 90% A and 10% B, after 0.5 minutes the proportion of B increased linearly to 40% until min. 2.5 and to 90% after 5.5 minutes. This concentration was kept constant until minute 6.8 and decreased again to 10 % after 7.5 minutes.

The instrument settings were as follows:

Nebulizing flow: 3 l/min; heating gas: 10 l/min; interface temperature: 390℃, DL temperature: 27℃; heat block temperature: 400℃, drying gas flow: 5 l/min.

The PEGs were measured in the positive multi reaction monitoring mode, also detected but not quantified were PEG 6, 7, 13, 14 and 15. In Table 1 the transitions for the PEGs 8 to 12 and the internal standard are shown.

A six-point calibration in water was performed for quantification. The calibration points used were 1, 2.5, 5, 10, 25 and 50 µg/ml.

Table 1. Multiple reaction monitoring used for quantification, the qualifiers and the retention time (RT). IS = Internal Standard

PEG	Target	Qualifier	RT (min)
PEG8	371>133	371>89	4,2
PEG9	415>133	415>89	4,72
PEG10	460>133	460>89	4,93
PEG11	503>133	503>89	5,12
PEG12	547>133	547>89	5,29
IS	223>103	223>59	4,47

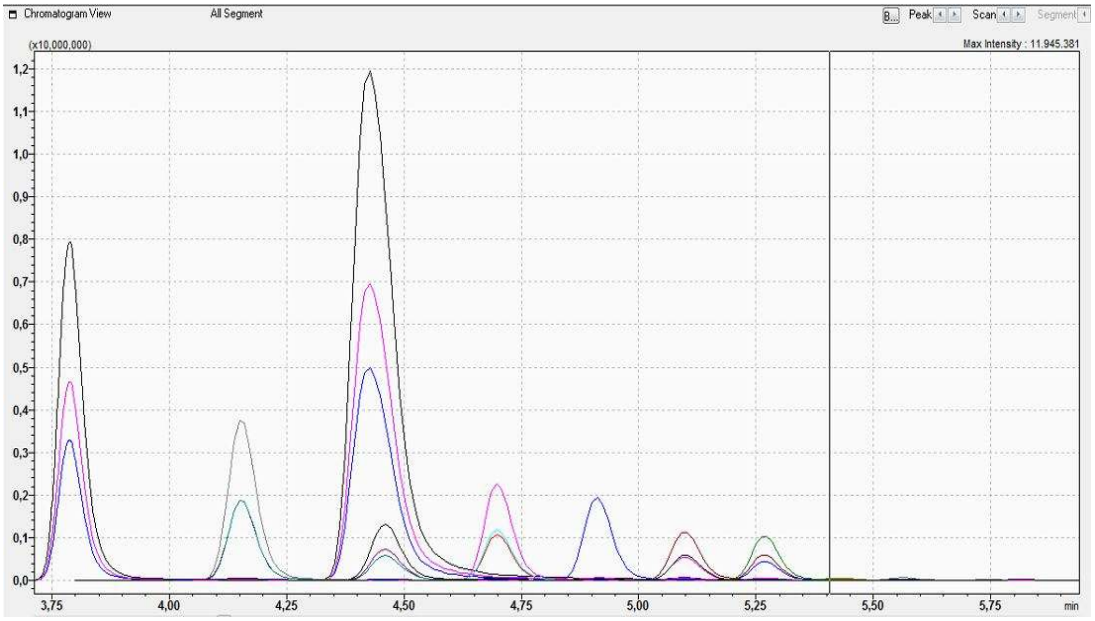

Fig. 1. Chromatogram of a PEG 6 to PEG 12 mixture with a concentration of 50 µg/ml each

2.2.2 Sample preparation

10 µl of the sample or calibrating solution dissolved in urine were mixed in an Eppendorf tube with 10 µl of a 100 µg/ml solution of dimethoxy-tetraethyleneglycol as internal standard, 10 µl of a 0.1 aqueous m zinc-sulfate and 40 µl water. The solution was thoroughly mixed and centrifuged after 5 min of cooling at 5 to 8℃. 10 ml of the supernatant were filled to an autosampler vial containing 1 ml of water. 5 µl of the sample were injected for analysis.

2.3 Statistics

If not otherwise stated, data are presented as means ± standard deviation. With the exception of the first 180 minutes, the most recent samples to minutes 400, 700, 1000, and 1300 after capsule ingestion, respectively, were included for kinetic analysis. The total excretion of PEG was individually calculated as the sum of all sample volumes multiplied by the appropriate PEG concentration. In order to avoid false positive marker detections, a PEG-concentration was regarded as positive if it exceeded the mean individual baseline concentration of PEG 8, PEG 9, PEG 10, PEG 11, and PEG 12 added by the threefold of standard deviation. Significant differences from baseline values were determined by One-way ANOVA, level of significance was set to $P \leq 0.05$. All statistics were performed using SPSS (Chicago, USA).

3. RESULTS

The individual 24 h drink volume ranged from 1.1 L to 6.4 L. 16 subjects consumed alcohol with the maximum of 180 g. There was a strong correlation between the total volumes of drinks and urines. The total 24 h urine volumes amounted to 1.98 ± 1.05 L with a minimum of 0.37 L and a maximum of 5.78 L. The PEG baseline concentrations were 0.41 ± 0.44 µg/ml, 0.36 ± 0.29 µg/ml, 0.43 ± 0.37µg/ml, 0.76 ± 0.81 µg/ml, and 0.59 ± 0.61 µg/ml for PEG8, PEG10, and PEG12, respectively. Peak-concentrations interindividually showed a wide range with maximal values between 40 and 1914 µg/ml for PEG8, between 46 and 1710 µg/ml for PEG10, and between 178 and 2066 µg/ml for PEG12. Fig. 2 depicts a nonlinear, reciprocal correlation between urine flow at the time of peak-concentration and the corresponding PEG concentrations.

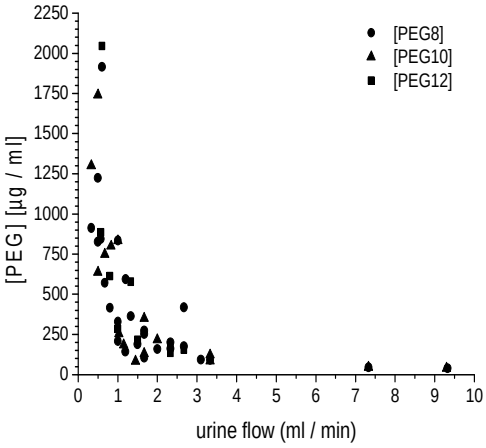

Fig. 2. Correlation between urine flow at individual PEG peak concentration and the corresponding concentrations of PEG 8, PEG 10, and PEG 12

The time courses of PEG concentrations are presented in Fig. 3. Significant differences to baseline values occurred from 30 minutes to 7 hours after marker ingestion (P = 0,05).

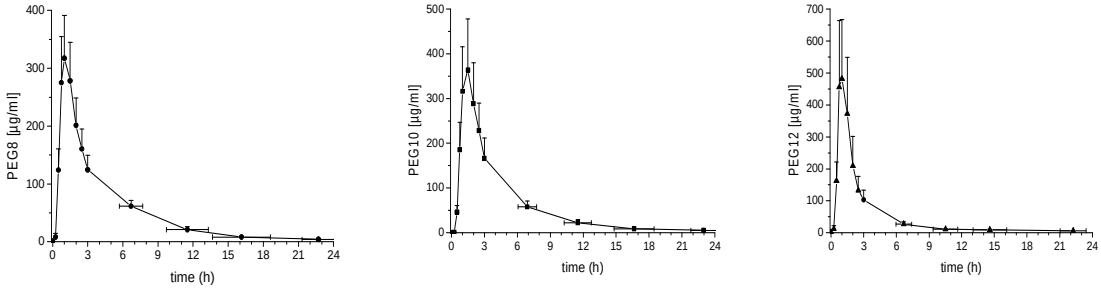

Fig. 3. Time courses of PEG8 concentration (n = 26, left), PEG10 concentration (n = 17, middle), and PEG12 concentration (n = 9, right)

Mean ± SE

Samples of all subjects were above threshold after 60 minutes. At the latest 22 hours following intake, the marker re-attained baseline values in all participants. Fig. 4 shows the initial 180 minutes after ingestion for all PEGs. Data are computed as percentage of the individual maximum.

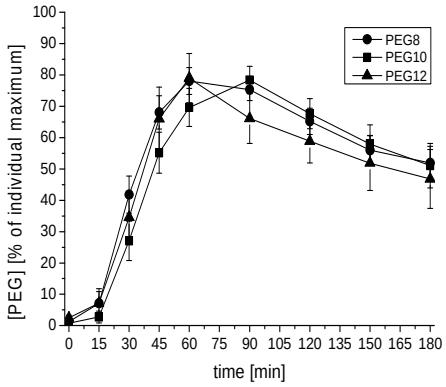

Fig. 4. Comparison of time courses of PEG8, PEG10, and PEG12 concentrations for the initial 180 minutes. Individual data are computed as percent of their maximal concentration

(Mean ± SE, n = 26 for PEG8, n = 17 for PEG10, n = 9 for PEG12)

The total excretion of PEGs averaged 144 ± 42.7 mg corresponding to 48 ± 14% of the intake.

4. DISCUSSION

PEGs of low molecular weight could be detected in the urine of all subjects even prior to marker ingestion. That is not surprising since PEGs are used for years as a galenic medium e.g. for drugs and they are regarded as inactive ingredients by the US Food and Drug Administration. Therefore, in order to prevent false-positive samples, we computed an individual threshold by means of the baseline urine samples. Unfortunately, this procedure can hardly be used in routine care. Since all baseline concentrations from PEG6 to PEG15 were in the same range, for routine administration we recommend an individual threshold computed by means of the three lowest PEG concentrations.

Our subjects were not supervised for the main part of the experimental phase and, therefore, the quality of results partly depended on their willingness to participate. The strong correlation between total fluid intakes and urine volumes may indicate a good cooperation concerning volume recording. However, this aspect did not affect the PEG marker analysis. Here, in all subjects the PEG urine marker was clearly identifiable, independently of alcohol consumption, total urine volume, and urine flow. But the statistically significant increases in marker concentrations already 30 minutes after oral intake may not mislead the fact that only after 60 minutes all subjects showed marker concentrations above threshold. This may explain the partial failure in the study of Jones et al. [7] to detect the PEG marker 30 to 40 minutes after ingestion. The authors reported a drop out of about 10% of samples. Peak values were obtained between minutes 60 and 90, marker concentrations then declined steadily until baseline values were reached 22 hours after ingestion in all subjects. Therefore, using different marker combinations, the method can be applied day by day in routine urine drug testing without interference with previously taken markers.

An average of 144 mg from 300 mg of the administered markers was excreted with the urine when baseline values were re-attained. Possible explanations are 1) that less than 50% of the substances were resorbed via the intestine, 2) a partial (transient) storage of PEG in intracellular compartments, and 3) a combination of 1) and 2). Since we did not measure PEG in faces, the answer remains speculative. However, the last explanation may be favored for two reasons. First, the urinary excretion of 60 % for PEG with M = 500 g x mo^{-1} [12], which is in the M-range of PEG used in the present study, is higher than the actual data. Secondly, in an early study, an intravenous injection of 1g low molecular PEG (M = 300 g x mol-1) resulted in a 77 % urinary excretion within the next 12 hours while a PEG with 20 times higher molecular mass was excreted by 96% [15].

5. CONCLUSION

The applied PEG markers could be detected in the urine of all subjects 60 minutes after oral capsule ingestion. Thus, if this delay is taken into account they are a promising, reliable, and easy to use tool to protect against manipulation of urine samples in the field of drug and doping control.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the

appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Allen KR. Screening for drugs of abuse: Which matrix, oral fluid or urine? Ann Clin Biochem. 2011;48:531–41.
2. Beck O, Carlsson S, Tusic M, Olsson R, Franzen L, Hulten P. Laboratory and clinical evaluation of on-site urine drug testing. Scand J Clin Lab Invest. 2014;74: 681-6.
3. Gerostamoulos D. Urinary drug screening. Aust Prescr. 2013;36:62-4.
4. Gudin JA, Mogali S, Jones JD, Comer SD. Risks, management, and monitoring of combination opioid, benzodiatepenes, and/or alcohol use. Postgrad Med. 2013; 125:115-130.
5. Möller MR, Steinmeyer S, Bregel S. The German approach to solving the problem to driving under the influence of drugs. Probl. Forensic Sci. 2000;42:152–9.
6. Tang M, Ching CK, Tse ML, Ng C, Lee C, Chong YK et al. Surveillance of emerging drugs of abuse in Hong Kong: Validation of an analytical tool. Hong Kong Med J. 2015;21:114–23.
7. Jones JD, Atchinson JJ, Madera G, Metz VE. Need and utility of a polyethylene glycol marker to ensure against urine falsification among heroin users. Drug Alcohol Depend. 2015;153:201–6.
8. Gauchel G, Huppertz B, Feiertag H, Keller R. Clinical use of polyethylene glycols as marker substances and determination in urine by liquid chromatography. J Chromatogr B. 2003;787:271–9.
9. Fruijtier-Pölloth C. Safety assessment on polyethylene glycols (PEGs) and their derivatives as used in cosmetic products. Toxicology. 2005;214:1-38.
10. EFSA. Opinion of the scientific panel of food additives, flavourings, processing aids and materials in contact with food on a request from the commission related to an application on the use of polyethylene glycol (PEG) as a film coatingagent for use in food supplement products. The EFSA Journal. 2006;414:1-22.
11. Gullapalli RP, Mazzitelli CL. Polyethylene glycols in oral and parenteral formulations – A critical review. Int J Pharm. 2015;496: 219-239.
12. Baumann A, Tuerck D, Prabhu S, Dickmann L, Sims J. Pharmacokinetics, metabolism and distribution of PEGs and PEGylated proteins: Quo vadis? Drug Discov Today. 2014;19:1623-1631.
13. Huppertz B, Gauchel G, Feiertag H, Schweizer H, Krieger H, Richter F, et al. Urine labeling with orally applied marker substances in drug substitution therapy. Clin Chem Lab Med. 2004;42:621-6.
14. Christensen K. Shifting the paradigm: A blueprint for value recognition and process improvement in the U.S. Drug-testing Market; 2014. Available:http://www.markertest.com/file.616-169783-2685/shifting-the-paradigm.pdf (Accessed 08.10.2017)
15. Shaffer CB, Critchfield H, Nair JH. The absorption and excretion of a liquid polyethylene glycol. J Pharm Sci. 1959;39: 340-344

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/22784