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Matrix Effects of Urine Marker Substances in LC-MS/MS Analysis of Drug of Abuse

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Background: Analysis of drug abuse is frequently performed using high-performance liquid chromatography with an MS/MS detector and electrospray ionization. In this context, matrix effects, like signal reduction by ion suppression of individual analytes, play an important role. In this study, the authors evaluated the matrix effect caused by polyethylene glycol (PEG) with chain lengths ranging from 6 to 12 repeating units in drug analysis by LC-MS/MS. Selected chain lengths were used in the Ruma urine marker system.

Methods and Results: Amphetamines, opiates, opioids, antidepressants, psychotics, benzodiazepines, z-substances, and individual drugs, including THCCOOH, cocaine, LSD, and some of their metabolites were investigated. The matrix effect was investigated at PEG concentrations of 500 mcg/mL and 20 mcg/mL. The effect of each PEG molecule was determined. Furthermore, the effects of different common sample preparations on the PEG matrix effects were evaluated. There was a strong correlation between the retention time of PEG and the drug that was ion-suppressed by PEG. The matrix effect decreased to the point where it was within an acceptable range at the lower PEG concentrations investigated in this study.

Conclusions: Matrix effects were observed for drugs with approximately the same retention times as the individual PEGs. The influence of the different workup methods was not as clear, which may be because of the similar solubilities of the PEGs and some analytes. At low PEG concentrations, the matrix effect was always below 60%, except for nortilidine. All the drugs were detectable. The effect on quantification was less than 15% for substances with deuterated analytes as internal standards and less than 32% for analytes without their own internal standards.

Key Words: matrix effects, LC-MS/MS analysis, polyethylene glycols, drug analysis

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INTRODUCTION

Triple-quadrupole mass spectrometry (MS) with upstream electrospray ionization is widely used in drug analysis. A disadvantage of this technique is matrix effects, which reduce the measurement signal owing to ion suppression or amplify the signal owing to increased ionization.[1,2] Especially the wide variability and high concentration levels of constituents in bioanalytical methods strongly affect electrospray ionization.[3] This was also observed for urine.[4] The influence of ionization type, sample preparation, and biofluid on bio-analysis of illicit drugs was investigated in 2003, using urine, oral fluid and plasma and by 4 sample preparation techniques, that is, direct injection and dilution only for urine and oral fluid, protein precipitation and solid phase extraction for all 3 biofluids.[5] According to the German Society of Toxicology and Forensic Chemistry (Gesellschaft für Toxikologische und Forensische Chemie, GTFCh) matrix effects must be smaller than ±25%.[6] Besides matrix effects, urine sample manipulation in drug-abusing clients poses a serious problem. One of the most efficient manipulations is the replacement of *drug-positive urine* with clean urine. A well-established routine to ensure the identity of urine samples without supervising the urination process is the application of polyethylene glycols (PEGs) of different chain lengths to mark the urine in vivo.[7,8] To this end, clients swallow PEGs with mean molecular weights of 300–600 Da (in chain length, PEG 6 to PEG 12) before urination. PEGs have been used for approximately 15 years in a liquid dosage form. More recently, PEGs for urine marking have become available as capsules contain PEG 7–11.[9] The use of capsules leads to significantly lower concentrations of PEGs in urine.[10] Therefore, this study aimed to determine the possible matrix effects of PEGs at different concentrations using a defined range of sample preparation methods.

MATERIALS AND METHODS

PEGs from the Ruma Marker System were used. They are available as liquids, which are used at relatively high PEG volumes, or as capsules, with significantly lower volumes.

A review of 10,000 urine samples marked with the Ruma Marker System showed a mean PEG concentration of approximately 800 mcg/mL for PEG 300 and 1500 mcg/mL PEG 600. Monodisperse PEGs were tested at a concentration

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The equipment and materials for this study were provided by the participating laboratories and were free of charge. The urine markers used in this study were provided by Ruma GmbH (Germany) and were free of charge.

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TABLE 1. MRM Transitions Used for Characterization of the Analytes and Level of Detection for Liquid/Liquid Extraction

Analyte	Precursor	Quantifier	Qualifier	LOD LLE ng/ml
THC-COOH	343	299	245	6.1
THCCOOH-D9	352	308	254	
EDDP	278	234	249/219	0.13
Morphine	286	152	201	1.2
Morphine-D3	289	165	153	
Codeine	300	199	215	0.76
Codeine-D6	306	218	165	
Dihydrocodeine	302	199	171	0.76
6-Monoacetylmorphine	328	211	168	1.75
Acetylcodeine	342	225	197/282	0.3
Noscapine	414	220	353	3.51
Papaverine	340	202	171	1.83
Cocaine	304	182	105	0.5
Benzoylecgonine	290	165	105	1.65
Benzoylecgonine-D8	298	171	110	
Amphetamine	136	91	119	0.65
Metamphetamine	150	91	119	0.41
Metamphetamine-D11	161	97	127	
MDA	180	163	135	0.19
MDMA	194	163	105	0.13
MDEA	208	163	135	0.11
PMA	165	149	121	1.19
PMMA	180	121	149	1.11
MBDB	208	135	77	0.98
Pregabaline	160	142	97	500
Cathinone	150	132	117	1.57
MDPV	276	126	135	0.97
Ketamine	238	125	207	2.12
Norketamine	224	125	179	0.81
Nordazepam	271	140	208	1.26
Oxazepam	287	241	104	0.3
Oxazepam-D5	292	246	274	
Temazepam	301	255	283	0.93
Diazepam	285	154	193	0.5
Alprazolam	309	281	205	2.99
OH-Alprazolam	325	297	216	2.84
OH-Alprazolam-D5	330	302		
Amino-Flunitrazepam	284	135	227	0.88
Amino-Flunitrazep.-D7	291	138		
Flunitrazepam	314	268	239	2.55
Nitrazepam	282	236	180	4.32
7-NH-Nitrazepam	252	121	94	2.14
Lorazepam	321	275	229	0.65
Lorazepam-D4	325	233		
Clonazepam	316	270	214	0.85
7-NH-Clonazepam	286	250		1.42
Zaleplon	306	264	236/219	3.98
Zoplicon	389	245	112	2.02
Zoplicon-D4	393	245		
Bromazepam	316	182	208	3.64
OH-Bromazepam	332	287	315	5.88

TABLE 1. (*Continued*) MRM Transitions Used for Characterization of the Analytes and Level of Detection for Liquid/Liquid Extraction

OH-Midazolam	342	324	168	2.09
Aminonitrazepam	252	121	94	2.14
OH-Triazolam	359	331	239/250	0.47
Clobazam	301	259	224	2.23
Desmethylclobazam	287	245	210/181	3.37
Flurazepam	388	315	317	1.21
Desalkylflurazepam	289	140	226/165	1.79
Norchlordiazepoxid	286	269	165/241	4.33
Prazepam	325	271	140	1.15
Fentanyl	337	188	105	0.05
Norfentanyl	233	84	55	0.19
Sufentanil	387	238	355	3.17
Norbuprenorphine	414	187	101	0.65
Norbuprenorphin-D3	417	187		
Buprenorphine	468	55	396	0.19
Buprenorphine-D4	472	59		
Hydrocodone	300	199	171	0.09
Oxycodone	316	298	241	0.73
Hydomorphone	286	185	157	0.18
Oxymorphone	302	227	284	4.55
Dihydromorphine	288	185	128	0.18
Tilidine	274	155	77	0.1
Nortilidine	260	155	229	0.67
LSD	324	223	207/281	0.96
2-Oxo-3OH-LSD	356	237	265	1.0
PCP	244	91	159	2.93
Psilocin	205	59	160	5.52
Ritalinic acid	220	84	56	62.7
Mescaline	212	195	180	4.62
Sertraline	306	275	159	0.93
PEG 6	283	89	133	200
PEG 7	327	89	133	200
PEG 8	371	89	133	200
PEG 9	415	89	133	200
PEG 10	459	89	133	200
PEG 11	503	89	133	200
PEG 12	574	89	133	200
PEG-IS	223	103	59	

of 500 mcg/mL to measure the influence of each PEG. PEGs with chain lengths of 6–12 repeating units were used in this study.

Different workup methods were applied to determine whether the matrix effects could be reduced by certain procedures. The methods used were liquid/liquid extraction (LLE), magnetic beads (Magtivio, Nuth, The Netherlands), solid-phase extraction (SPE), and protein precipitation with dilution (PD). To determine the effect of PEG concentration, different work-up methods were tested with a mixture of PEG 6–12 at a concentration of 20 mcg/mL. The effect of the PEGs on quantification was tested with PEG 6–12 at 500 mcg/mL and 20 mcg/mL after LLE.

All the solvents used were of LC-MS grade (Carl Roth, Karlsruhe, Germany). Standards were purchased from Cerilliant (Round Rock, TX).

For the experiments, a low concentration of addictive substances within the range required by the German catalog for chemical-toxicological analyses of fitness to drive (CTU criteria)[11] was used to check whether these substances were still detectable despite any matrix effect. The PEGs were applied at concentrations roughly corresponding to the concentration of Ruma liquid markers, as per the data collected from approximately 10,000 actual samples, and a concentration typical of urine after Ruma capsule ingestion.

The analysis was performed using a Shimadzu LC-MS 8050 instrument (Shimadzu, Kyoto, Japan). The sample (5 μL) was injected onto a Restek biphenyl column (Restek, Bellefonte, PA) 150·3 mm 2.7 μL and separated with a water–methanol gradient containing 0.1% formic acid and 0.002 mol/L ammonium formate. Eluent A contained 100% water and eluent B 100% methanol. The column was first

flushed with 10% eluent B for 0.5 minutes. Thereafter, as a linear gradient, the percentage of eluent B was increased in the first step to 40% at 2.5 minutes, in the second step to 90% B after 5.5 minutes, and held for 8.5 minutes. After 9 minutes, the eluent contained 10% B again until the gradient ended at 10 minutes. The flow decreased from 0.35 mL/min at the beginning to 0.2 mL/min after 9 minutes. In compliance with ISO 17025, the method's target analytes were detected in MRM mode with at least 2 transitions. The MRMs of all tested analytes and the limits of detection after liquid/liquid extraction are summarized in Table 1.

Liquid/liquid extractions were performed using 100 μL of the sample. The sample was shaken with saturated saline (1.5 mL of saturated saline) mixed with 2 mL of ethyl acetate/diethyl ether (1:1). The mixture was mixed in an overhead mixer for 10 minutes and centrifuged at 1293*g* for 5 minutes. The supernatant was then transferred to another glass tube, evaporated, and dissolved in methanol/water (1:3). Five microliters of this mixture were injected into the LC-MS system.[12]

Magnetic beads are cleaning systems that were developed by Magtivio for analysis of urine and blood samples. Fifty microliters of urine were mixed with 40 μL of a suspension containing magnetic beads in an Eppendorf cup and then a precipitation reagent out of 2 components (150 μL) was added to the urine/beads mixture and shaken well. The vial was placed on a magnetic holder for 2 minutes and the supernatant was pipetted into an autosampler vial for analysis. The Magtivio magnetic bead system removes unwanted ingredients such as salts, creatinine, and proteins from the sample, and the cleaned urine with the analytes remained. Five microliters of the clear solution were injected.

For protein precipitation, 400 μL of acetonitrile was added to 100 μL of the sample and centrifuged at 12,298*g* for 10 minutes in Eppendorf cups. Five microliters of the supernatant were injected.

Bond Elute Certify columns from Agilent, Santa Clara, CA, designed specifically for drug analysis (DAO), were used for SPE. The cartridges were conditioned with 2 mL of methanol followed by 2 mL of 0.1 M phosphate buffer pH 6, then 100 μL of sample was diluted to 1 mL with water and spiked with 700 μL of phosphate buffer pH 6. The column was then rinsed with 6 mL of water and 1 mL of 0.1 M acetic acid. The column was dried under vacuum for 20 minutes. After adding 100 μL of methanol, the column was dried again under vacuum. The drugs of abuse were eluted first with 2 mL of ethyl acetate/NH_4OH 25% (98/2 v/v) (SPE 1), followed by 2 mL of dichloromethanes/isopropanol/NH_4OH 25% (80/20/2 v/v/v) (SPE 2). Eluates were collected and dried under a stream of nitrogen. The residue was dissolved in water–methanol 1/3 v/v and 5 μL were injected into the LC-MS/MS system.

Forensic bioanalytical assays involving magnetic beads and liquid/liquid extraction are in accordance with ISO/IEC 17025.

For each method, 5 urine samples were mixed with narcotic substances at 1 concentration and analyzed. Peak areas were determined, and the mean values were calculated. Subsequently, the same 5 urine samples were spiked with the same amounts of narcotics and PEGs. The experiments were performed using different marker concentrations. Mixtures of PEG 6–12 or individual PEGs were added to narcotic-spiked urine. The mean peak areas of the narcotics were compared with and without PEG. The matrix effect was obtained from this difference. This was expressed as a percentage decrease or increase in the peak area.

In another experiment, the influence of PEGs on the quantification was investigated. For this purpose, a drug-spiked sample was prepared using liquid/liquid extraction and quantified. Then, 3 aliquots were spiked with 20 mcg/mL or 500 mcg/mL PEG 6–12 or 500 mcg/mL PEG 7–12, respectively, and processed and quantified in the same manner.

RESULTS

The first experiments were performed with urine samples spiked with drugs and with 500 mcg/mL of each PEG 6–12 separately to evaluate which PEG chain length was responsible for the ion suppression of the corresponding analytes. Next, we tested the influence of different sample preparation methods on the matrix effect. The influence of the PEG concentration was measured in another test using LLE for sample preparation. LLE was used to evaluate the effect of PEG on the analyte quantification. A mixture of PEG 6–12 was used in the last 3 experiments.

Matrix effects are related to substances that have retention times similar to the retention times of the individual markers. Matrix effects were mostly correlated with marker concentration. Analytes eluted before the PEGs or only afterward did not show matrix effects above the 25% that the German Society of Toxicology and Forensic Chemistry (GTFCh) 6 provides as a tolerance limit for forensic analytical methods in its guidelines for validation of forensically safe methods.

At marker concentrations of 500 mcg/mL PEG 6–12, no matrix effect >25% was detected for the following substances, regardless of sample preparation: THCCOOH, diazepam, nordazepam, oxazepam, temazepam, prazepam, OH-alprazolam, desalkylflurazepam, lorazepam, clobazam, desmethylclobazam, OH-midazolam, bromazepam, flunitrazepam, clonazepam, zaleplon, sertraline, EDDP, hydromorphone, morphine, pregabalin, cathinone, amphetamine, and methamphetamine. Not all analytes can be detected by every working-up method depending on their solubility. For evaluation, these analytes were not included in the tables.

With liquid/liquid extraction as sample preparation, a good correlation between the matrix effects and the retention times of drugs and PEGs 6–11 was shown. Table 2 presents an overview of the substances affected by matrix effects and the PEG chain length responsible for ion suppression. The effect was particularly strong, with approximately 90% for MDMA, hydrocodone, ritalinic acid, cocaine, 6-acetylcodein, norbuprenorphine, tilidine, 7-amino-flunitrazepam, LSD, and tramadol. Owing to its strong signal and high concentration, tramadol was still detectable with sufficient sensitivity. For LSD norbuprenorphine, 7-amino-clonazepam, fentanyl, and tilidine, a

TABLE 2. Matrix Effect Measured With LC-MS After Liquid/Liquid Extraction

		PEG 6	PEG 7	PEG 8	PEG 9	PEG 10	PEG 11	PEG 12
	RT	4.13	4.61	4.91	5.26	5.47	5.69	5.99
Oxycodone	4.12	−58.34	−21.81	−7.59	−10.63	−17.50	−8.87	−35.91
MDMA	4.16	−94.98	−31.23	−16.33	−15.63	−16.83	−8.42	−59.11
PMMA	4.25	−87.35	−28.82	−15.84	−16.44	−19.79	−12.62	−48.80
Hydrocodone	4.26	−91.62	−26.62	−9.02	−13.25	−17.98	−8.68	−47.75
2-Oxo-3-OH-LSD	4.29	−57.54	−17.80	−8.30	−6.09	−14.66	−4.67	−26.32
MDE	4.51	−20.26	−50.35	−9.43	−2.55	−8.85	4.20	−20.22
Ritalinic acid	4.6	−22.47	−94.31	−25.56	−15.54	−19.49	−11.84	−39.70
MBDB	4.71	−4.63	−69.54	−14.08	−9.96	−17.04	−8.04	−26.12
Norfentanyl	4.75	−0.52	−43.92	−4.54	−6.19	−12.94	−1.42	−18.90
Ketamine	4.78	7.14	−19.45	−35.18	−8.28	−10.90	0.01	−13.69
Tramadol	4.87	−14.48	−44.89	−95.21	−31.17	−26.13	−12.39	−50.97
N-Desmethyl-tramadol	5.04	0.25	−30.60	−69.92	−10.89	−14.94	0.64	−28.96
Benzoylecgonine	5.1	2.39	−23.06	−52.54	−9.65	−17.19	−6.34	−20.70
6-Acetylcodeine	5.17	−0.24	−26.14	−34.08	−96.56	−38.14	−13.09	−41.89
Nortilidine	5.17	5.67	−24.64	−36.02	−94.87	−34.25	−9.56	−40.46
Cocaine	5.28	1.47	−26.96	−32.37	−86.58	−29.83	−9.46	−38.51
Zopiclon	5.28	−1.98	−20.67	−33.74	−83.69	−24.48	6.16	−27.88
7-NH-Clonazepam	5.35	10.24	−9.67	−1.24	−34.91	−19.49	1.59	−10.35
Tilidine	5.41	7.95	−17.19	−17.66	−30.47	−95.63	−40.54	−39.01
Norbuprenor-phine	5.45	4.28	−20.00	−11.92	−24.73	−92.60	−31.29	−26.60
LSD	5.55	4.26	−22.16	−34.76	−30.19	−87.71	−31.54	−37.94
7-NH-Flunitrazepam	5.93	4.11	−18.97	2.03	−3.03	−27.94	−42.87	−90.89
Fentanyl	5.99	−3.81	−24.74	−7.71	−6.53	−32.00	−31.06	−69.82
Buprenorphine	6	3.48	v23.18	−0.61	1.50	−22.47	−12.13	−55.80

metabolite or parent substance, can be used for consumption detection. PEG 11 did not cause matrix effects on any of the substances investigated. For PEG 12, matrix effects below 50% but greater than 25% were observed for many analytes, largely independent of retention time and also with different sample preparation methods.

The correlations between sample preparation and matrix effects are shown in Table 3. For LLE, magnetic beads, protein precipitation and dilution, and solid-phase extraction of the first and second elution steps, the matrix effect was comparable for most analytes.

Table 4 shows the influence of PEG concentration on the PEG caused matrix effect after sample preparation with liquid/liquid extraction and the SD between the 5 measurements of each PEG concentration. The highest concentration was found only after the intake of the liquid marker (500 mcg/mL of PEG 6–12 combined), and 20 mcg was half of the medium concentration found after the intake of the marker capsules (evaluation of approximately 1000 urine samples after capsule intake). One-third of these urine samples showed PEG concentrations of 20 mcg/mL or less. Most analytes showed decreasing matrix effects with decreasing PEG concentrations.

TABLE 3. Influence of Different Sample Preparations on Matrix Effects on Drug Analysis With LC-MS

Analyte	RT	LLE	Beads	PD	SPE 2nd Ext.	SPE1 1st Ext.
Oxycodone	4.12	−51.5	58.3	−84.8	82.0	10.4
MDMA	4.16	−85.9	95.0	−93.8	97.8	18.2
PMMA	4.25	−76.7	87.4	−84.3	92.6	43.8
Hydrocodone	4.26	−79.0	91.6	−85.3	91.6	28.7
2-Oxo-3-OH-LSD	4.29	−45.7	57.5	48.8	77.8	32.1
MDE	4.51	−47.8	50.4	35.7	87.9	3.7
Ritalinic acid	4.6	−82.1	94.3	−93.3	83.8	47.9
7-NH-Nitrazepam	4.61	−86.9		−92.3	81.7	25.5
MBDB	4.71	−58.1	69.5	−58.8	88.8	5.0
Norfentanyl	4.75	−38.1	43.9	−37.5	71.2	34.6
Ketamine	4.78	−47.8	35.2	−35.2	78.6	17.7
Tramadol	4.87	−82.1	95.2	−94.5	94.9	19.6
N-Desmethyltramadol	5.04	−86.9	69.9	−72.3	71.4	13.6
Benzoylecgonine	5.1	−58.1	52.5	−57.6	81.1	31.7
6-Acetylcodeine	5.17	−91.0	96.6	−96.0	80.8	n.d.
Nortilidine	5.17	−90.8	94.9	−93.4	86.2	45.5
Cocaine	5.28	−77.0	86.6	−85.6	85.0	70.7
Zopiclon	5.28	−76.6	83.7	−83.6	60.1	n.d.
7-NH-Clonazepam	5.35	−85.1	34.9	−51.2	40.3	4.3
MDPV	5.4	−89.5		−85.2	87.1	3.7
Tilidine	5.41	−91.2	96.6	−94.3	85.0	7.1
Norbuprenorphine	5.45	83.3	92.6		65.4	3.1
LSD	5.55	−48.0	87.7	−87.9	80.7	57.5
7-NH-Flunitrazepam	5.93	−78.6	90.9	−93.4	50.4	62.3
Fentanyl	5.99	−69.4	69.8	−72.6	66.7	51.0
Buprenorphine	6	44.5	55.8	−68.9	40.7	8.3

Influence of different sample preparations on the matrix effect of PEG 6–12 on drug analysis with LC-MS. The concentration of each PEG is 500 mcg/mL. The drug concentration is 50 ng/mL except for LSD, fentanyl, and buprenorphine, and metabolite with a concentration of 5 ng/mL and ritalinic acid 200 ng/mL, n.d.: not detectable. The headers represent LLE for liquid/liquid extraction. Beads for sample cleaning with magnetic beads; StrHe for precipitation and dilution, and SPE for solid phase extraction.

The matrix effect decreased by more than 25% for most of the analytes.

Oxycodone, 2-oxo-3-hydroxy-LSD, MDE, norfentanyl, ketamine, desmethyltramadol, benzoylecgonine, and 7-aminoclonazepame mostly showed matrix effects well below 25% at a PEG concentration of 20 mcg/mL in contrast with higher matrix effects with sometimes more than 50% at 500 mcg/mL PEG.

Table 5 compares the concentration of the analytes in urine without PEG to concentrations present with 20 mcg/mL or 500 mcg/mL of PEG 6, 7, 8, 9, 10, 11, and 12 each and 500 mcg/mL of each PEG 7–12 for specific analytes that show ion suppression in presence of PEG 6. The deviations are indicated as percentages. The chosen lower PEG concentration of 20 mcg/mL did not represent the mean PEG concentration used in capsules for the Ruma Marker-System investigated in this study. One-third of urine samples showed significantly lower concentrations after capsule ingestion. However, even at this relatively high concentration, the matrix effects were only above 25% for the 3 analytes, namely MDMA, hydrocodone, and LSD. Individual deuterated substances as internal standards were not used for any of the analytes. MDA-d5

TABLE 4. Influence of the PEG Concentration on Matrix Effects at Drug Analysis With LC-MS

		PEG concentration			
Analyte	**RT**	**500 µg/ml**	**SD%**	**20 µg/ml**	**SD%**
Oxycodone	4.12	−51.5	10.8	−12.0	11.7
MDMA	4.16	−85.9	15.3	−54.0	6.5
PMMA	4.25	−76.7	11.5	−44.0	6.4
Hydrocodone	4.26	−79.0	8.5	−38.0	7.6
2-Oxo-3-OH-LSD	4.29	−45.7	10.1	−4.6	15.0
MDE	4.51	−47.8	8.8	−2.7	11.1
Ritalinic acid	4.6	−82.1	10.5	−52.2	20.6
7-NH-Nitrazepam	4.61	−86.9	15.0	−55.5	9.8
MBDB	4.71	−58.1	9.8	−52.1	10.0
Norfentanyl	4.75	−38.1	8.8	24.6	9.4
Ketamine	4.78	−47.8	10.2	11.0	7.4
Tramadol	4.87	−82.1	11.9	−45.1	9.4
N-Desmethyltramadol	5.04	−86.9	16.1	2.8	7.6
Benzoylecgonine	5.1	−58.1	14.8	3.3	5.5
6-Acetylcodeine	5.17	−91.0	7.6	−45.3	5.6
Nortilidine	5.17	−90.8	17.6	−77.2	2.7
Cocaine	5.28	−77.0	12.8	−30.8	5.4
Zopiclon	5.28	−76.6	11.7	−31.9	34.5
7-NH-Clonazepam	5.35	−85.1	15.0	−0.4	14.8
MDPV	5.4	−89.5	11.8	−40.1	13.4
Tilidine	5.41	−91.2	10.3	−54.6	13.1
Norbuprenorphine	5.45	−83.3	12.9	−57.1	8.2
LSD	5.55	−48.0	13.5	−25.1	9.5
7-NH-Flunitrazepam	5.93	−78.6	13.5	−58.0	8.4
Fentanyl	5.99	−69.4	9.0	−34.7	5.3
Buprenorphine	6	44.5	10.6	−26.0	4.9

In yellow are the results of 500 mcg/mL of each PEG and in blue 20 mcg/mL of each PEG. Additionally, the SD of 5 analyses is shown for each analyte.

was used as internal standard (IS) for MDMA, codeine-d6 for hydrocodone, and NH-flunitrazepam-d5 for LSD. The investigated system did not use PEG 6 in their capsules. For semiquantitative analytes such as the analytes that have no specific internal standard, a deviation of ±30% is acceptable, as per the cited GTFCh guidelines. The quantitative analytes marked with x for specific individual internal standards showed less than 15% deviation from the urine without PEG. The effect of PEG on the quantification of buprenorphine was low at only 4%, even with 500 mcg/mL PEG assumed as a typical

TABLE 5. Influence of PEG Concentration and Effect on Quantification

	RT	IS	No PEG c / ng/ml	20µg PEG 6–12 % dev.	500 µg PEG 6–12 % dev.	500 µg PEG 7–12 % dev.
Oxycodone	4.05		60.4	−19.3	−57.4	−14.5
MDMA	4.10		78.4	−39.0	−72.2	−23.1
Hydrocodone	4.11		72.4	−29.9	−67.2	−22.8
2-Oxo-3-OH-LSD	4.23		5.8	−20.9	−50.2	−7.2
MDE	4.40		56.9	−17.3	−41.8	−47.5
7-NH-Nitrazepam	4.56		56.1	13.6	59.9	
Norfentanyl	4.67		5.6	−16.5	−36.0	
Tramadol	4.81		51.4	−16.8	−62.4	
N-Desmethyltramadol	4.93		43.9	24.5	249.6	
Benzoylecgonine	5.00	x	53.6	−2.0	20.4	
6-Acetylcodeine	5.10		71.5	−20.6	−46.9	
Nortilidine	5.12		64.3	−16.4	−9.7	
Zopiclon	5.22	x	61.3	−12.4	16.5	
7-NH-Clonazepam	5.31		133.3	−18.0	−55.5	
Tilidine	5.34		64.0	−15.5	−37.7	
Norbuprenorphine	5.38	x	5.5	−12.6	−25.8	
LSD	5.43		8.4	−31.0	−49.8	
7-NH-Flunitrazepam	5.86	x	48.3	11.7	78.3	
Fentanyl	5.91		6.4	−20.3	−12.3	
Buprenorphine	5.92	x	5.6	0.8	4.4	
Noscapine	6.01		46.3	−6.0	−42.9	
OH-Bromazepam	6.07		72.8	−8.9	−38.5	

Influence of 20 mcg/mL and 500 mcg/mL of each PEG in a combination of PEG (6–12) and 500 mcg/mL PEG 7–12 on quantification. Different colors of RT markers the different PEG from 6 to 12 repeating units. Analytes that have the deuterated form as an internal standard (IS) are marked with an x.

concentration for liquid urine marker solutions. The deviation from the concentrations found in urine without PEG was higher for all other analytes.

For several analytes, the deviation was significant at low or high concentrations, depending on the effect of PEG on the internal standard.

DISCUSSION

In the guidelines of the German Society for Toxicology and Forensic Chemistry (GTFCh for the validation of forensic analysis methods, the matrix effect in an LC-MS method should be less than 25% for an analyte in 6 different spiked samples of 1 matrix, such as urine, oral liquid, or blood.6 The matrix effect can be caused by phospholipids, proteins, or other analytes. For more than 20 years, PEGs have been used in drug therapy as a marker system to replace urine under supervision. During this time, most of the forensic and confirmation analyses were performed using GC-MS involved matrix effects. Moreover, LC-MS has replaced GC-MS in drug abuse analyses because of significant sensitivity improvement, less effort in sample preparation, and less material requirement. However, matrix effects can be an issue in

LC-MS analysis, particularly at high concentrations of interfering substances. The PEG concentrations used for urine marking were in the milligram/mL range. Of 9500 urine samples investigated separately at the laboratory, the concentration of PEG 600 had a median concentration of 1.35 mg/mL and 99% of them were less than 14 mg/mL. For PEG 300, the median concentration was 1.1 mg/mL and 99% of them were less than 7.5 mg/mL. These are rather high concentrations in comparison to the concentration of the actual target analytes in urine, which are measured in ng/mL to mcg/mL. For our study, even a high concentration of 0.5 mg/mL of monodisperse PEG was used, comparable to a PEG 300 concentration of 2.5 mg/mL and a PEG 600 concentration of more than 5 mg/mL. Monodisperse PEGs (6–12) were tested individually and in combination. The drug concentration was chosen to be in the lower range of 50 ng/mL for most analytes, except for LSD, fentanyl, buprenorphin, THCCOOH, acetylcodeine, and 6-MAM, which had concentrations of 5 ng/mL. All analytes were detected even at high PEG concentrations. The matrix effect of the PEGs on each analyte depended on the retention time. There was a strong correlation between the retention time of the drug, matrix effects, and the retention time of PEG. The different sample preparations, liquid/liquid extraction, protein precipitation/dilution, magnetic beads, and solid-phase extraction had negligible influences on the matrix effects of the PEGs. This may indicate that the PEGs behaved similarly to the analytes. In addition, the retention times were within the range of several commonly used drugs. A significant matrix effect was detected for PEG 6 and MDMA, which resulted in a very small MDMA signal that could cause a negative result at MDMA concentrations of <50 ng/mL. The newly developed Ruma marker in capsules contained only 2 different monodisperse PEGs per dose, in contrast to the previously used polydisperse liquid marker. The amount of PEG used in the capsules, and thus the PEG concentrations measured in urine, were also lower than those measured with the liquid marker. The range of median urine concentrations after capsule intake ranges from 34 to 55 mcg/mL, and 90% of these were less than 100 mcg/mL. Approximately 350 urine samples containing PEGs 7–10 were tested. The use of capsules instead of polydisperse liquid markers can significantly reduce the matrix effects of PEGs. In this study, the influence of the PEG concentration on the matrix effects was demonstrated, as shown in table 4. Increasing the PEG concentration increased the matrix effect for most analytes. The effect was nearly negligible for the lower PEG concentration, but significant for most analytes at the higher PEG concentration of 500 mcg/mL for each PEG.

The effect of PEGs on the quantification of drugs of abuse strongly depends on the PEG concentration. At lower PEG concentrations, the effect on quantification was negligible for most analytes, particularly if they were deuterated as an internal standard. Higher PEG concentrations showed significant effects on the quantification by more than 25%, even for analytes with deuterated internal standards. For some analytes, higher concentrations were measured than those in the samples without PEG. This was caused by the signal suppression of the internal standard, which was higher than that of the analyte. Finally, the marker capsule had a significantly lower matrix effect than the liquid marker owing to its lower PEG concentration in urine.

CONCLUSION

As expected, the PEG matrix effects correlated well with drug retention time. Matrix effects were observed for drugs with approximately the same retention times as the individual PEGs.

The effects were particularly pronounced for PEGs 6–8 for the substance class of amphetamines and some opiates. PEG 6 was not included in the investigated marker capsules because it suppressed DMA detection.

The influence of the different workup methods was not as clear, which may be because of the similar solubilities of the PEGs and some analytes. Thus, it is expected that sample preparation that separates PEGs almost quantitatively also removes a considerable proportion of more water-soluble drugs from detection. Although matrix effects certainly occurred, given appropriate PEG concentrations and sample preparation, all substances remained detectable. Liquid markers with higher PEG concentrations are less suitable for LC-MS analysis.

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