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Alcohols, ketones and ethers – Determination of alcohols, ketones and ethers in urine by headspace GC-MS

Biomonitoring Method – Translation of the German version from 2020

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Abstract

The working group "Analyses in Biological Materials" of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area developed and verified the presented biomonitoring method.

The analytical method described hereinafter permits the simultaneous determination of various alcohols, ketones and ethers in urine. Methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, *tert*-butanol and isobutanol are determined in the group of alcohols. In the group of ketones, acetone, 2-butanone (methyl ethyl ketone), 2-pentanone, 3-pentanone, 3-methyl-2-butanone, cyclopentanone, 2-hexanone, 3-hexanone, 3,3-dimethyl-2-butanone (methyl-*tert*-butyl ketone), 4-methyl-2-pentanone (methyl isobutyl ketone), cyclohexanone, 2-heptanone, 3-heptanone and 4-heptanone are determined. In addition, this method can be used for the determination of the ethers methyl-*tert*-butyl ether (MTBE), tetrahydrofuran (THF) and 1,4-dioxane. For determination, the internal standards (ISTD) are added into gas-tight headspace vials containing the urine samples. The solutions are heated to 60 °C in the autosampler and then an aliquot of the headspace phase is transferred to the gas chromatograph and analysed by mass spectrometry. Calibration standards are prepared in water and processed in the same way as the samples to be analysed.

1 Characteristics of the method

Matrix Urine

Analytical principle Headspace GC-MS

Parameters and corresponding hazardous substances

Hazardous substance	CAS No.	Parameter	CAS No.
Alcohols			
Methanol	67-56-1	Methanol	67-56-1
Ethanol	64-17-5	Ethanol	64-17-5
1-Propanol	71-23-8	1-Propanol	71-23-8
2-Propanol	67-63-0	2-Propanol	67-63-0
		Acetone	67-64-1
1-Butanol	71-36-3	1-Butanol	71-36-3
2-Butanol	78-92-2	2-Butanol	78-92-2
tert-Butanol	75-65-0	*tert*-Butanol	75-65-0
Isobutanol (2-methyl-1-propanol)	78-83-1	Isobutanol	78-83-1
Ketones			
Acetone	67-64-1	Acetone	67-64-1
2-Butanone (methyl ethyl ketone)	78-93-3	2-Butanone	78-93-3
2-Pentanone	107-87-9	2-Pentanone	107-87-9
3-Pentanone	96-22-0	3-Pentanone	96-22-0
3-Methyl-2-butanone (Methyl isopropyl ketone)	563-80-4	3-Methyl-2-butanone	563-80-4
Cyclopentanone	120-92-3	Cyclopentanone	120-92-3
2-Hexanone	591-78-6	2-Hexanone	591-78-6
3-Hexanone	589-38-8	3-Hexanone	589-38-8
3,3-Dimethyl-2-butanone (methyl-*tert*-butyl ketone)	75-97-8	3,3-Dimethyl-2-butanone	75-97-8
4-Methyl-2-pentanone (methyl isobutyl ketone)	108-10-1	4-Methyl-2-pentanone	108-10-1
Cyclohexanone	108-94-1	Cyclohexanone	108-94-1
2-Heptanone	110-43-0	2-Heptanone	110-43-0
3-Heptanone	106-35-4	3-Heptanone	106-35-4
4-Heptanone	123-19-3	4-Heptanone	123-19-3
Ethers			
Methyl-*tert*-butyl ether (MTBE)	1634-04-4	MTBE	1634-04-4
		tert-Butanol	75-65-0
Tetrahydrofuran (THF)	109-99-9	THF	109-99-9
1,4-Dioxane	123-91-1	1,4-Dioxane	123-91-1

Reliability data

Methanol

Within-day precision:	Standard deviation (rel.)	s_w = 6.2% or 2.3%
	Prognostic range	u = 14.0% or 5.2%
	at a spiked concentration of 2.9 mg or 29.4 mg methanol per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 2.9% or 3.1%
	Prognostic range	u = 7.0% or 7.3%
	at a spiked concentration of 2.9 mg or 29.4 mg methanol per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 94.3% or 98.5%
	at a spiked concentration of 2.9 mg or 29.4 mg methanol per litre urine and where n = 10 determinations	
Detection limit:	0.2 mg methanol per litre urine	
Quantitation limit:	0.6 mg methanol per litre urine	

Ethanol

Within-day precision:	Standard deviation (rel.)	s_w = 4.6% or 2.2%
	Prognostic range	u = 10.4% or 5.0%
	at a spiked concentration of 3.0 mg or 30.2 mg ethanol per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 2.7% or 2.6%
	Prognostic range	u = 6.5% or 6.2%
	at a spiked concentration of 3.0 mg or 30.2 mg ethanol per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 91.0% or 101%
	at a spiked concentration of 3.0 mg or 30.2 mg ethanol per litre urine and where n = 10 determinations	
Detection limit:	0.1 mg ethanol per litre urine	
Quantitation limit:	0.3 mg ethanol per litre urine	

1-Propanol

Within-day precision:	Standard deviation (rel.)	s_w = 9.7% or 2.4%
	Prognostic range	u = 21.9% or 5.4%
	at a spiked concentration of 0.5 mg or 5.3 mg 1-propanol per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 9.1% or 4.1%
	Prognostic range	u = 21.4% or 9.8%
	at a spiked concentration of 0.5 mg or 5.3 mg 1-propanol per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 91.7% or 97.6%
	at a spiked concentration of 0.5 mg or 5.3 mg 1-propanol per litre urine and where n = 10 determinations	
Detection limit:	0.03 mg 1-propanol per litre urine	
Quantitation limit:	0.09 mg 1-propanol per litre urine	

2-Propanol

Within-day precision:	Standard deviation (rel.)	s_w = 2.5% or 1.8%
	Prognostic range	u = 5.7% or 4.0%
	at a spiked concentration of 0.5 mg or 5.4 mg 2-propanol per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 4.8% or 1.4%
	Prognostic range	u = 11.3% or 3.2%
	at a spiked concentration of 0.5 mg or 5.4 mg 2-propanol per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 98.3% or 98.7%
	at a spiked concentration of 0.5 mg or 5.4 mg 2-propanol per litre urine and where n = 10 determinations	
Detection limit:	0.02 mg 2-propanol per litre urine	
Quantitation limit:	0.06 mg 2-propanol per litre urine	

1-Butanol

Within-day precision:	Standard deviation (rel.)	s_w = 8.1% or 2.2%
	Prognostic range	u = 18.4% or 5.0%
	at a spiked concentration of 1.0 mg or 9.9 mg 1-butanol per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 10.2% or 4.4%
	Prognostic range	u = 24.2% or 10.5%
	at a spiked concentration of 1.0 mg or 9.9 mg 1-butanol per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 77.0% or 98.6%
	at a spiked concentration of 1.0 mg or 9.9 mg 1-butanol per litre urine and where n = 10 determinations	
Detection limit:	0.10 mg 1-butanol per litre urine	
Quantitation limit:	0.30 mg 1-butanol per litre urine	

2-Butanol

Within-day precision:	Standard deviation (rel.)	s_w = 6.2% or 1.8%
	Prognostic range	u = 14.0% or 4.1%
	at a spiked concentration of 0.5 mg or 5.3 mg 2-butanol per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 5.4% or 3.8%
	Prognostic range	u = 12.8% or 9.0%
	at a spiked concentration of 0.5 mg or 5.3 mg 2-butanol per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 91.9% or 94.1%
	at a spiked concentration of 0.5 mg or 5.3 mg 2-butanol per litre urine and where n = 10 determinations	
Detection limit:	0.05 mg 2-butanol per litre urine	
Quantitation limit:	0.15 mg 2-butanol per litre urine	

tert-Butanol

Within-day precision:	Standard deviation (rel.)	s_w = 7.3% or 2.2%
	Prognostic range	u = 16.6% or 4.9%
	at a spiked concentration of 0.5 mg or 4.9 mg *tert*-butanol per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 3.2% or 4.0%
	Prognostic range	u = 7.5% or 9.4%
	at a spiked concentration of 0.5 mg or 4.9 mg *tert*-butanol per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 93.9% or 93.8%
	at a spiked concentration of 0.5 mg or 4.9 mg *tert*-butanol per litre urine and where n = 10 determinations	
Detection limit:	0.05 mg *tert*-butanol per litre urine	
Quantitation limit:	0.15 mg *tert*-butanol per litre urine	

Isobutanol

Within-day precision:	Standard deviation (rel.)	s_w = 3.6% or 3.2%
	Prognostic range	u = 8.0% or 7.4%
	at a spiked concentration of 0.5 mg or 5.2 mg isobutanol per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 4.8% or 2.6%
	Prognostic range	u = 11.3% or 6.2%
	at a spiked concentration of 0.5 mg or 5.2 mg isobutanol per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 102% or 95.8%
	at a spiked concentration of 0.5 mg or 5.2 mg isobutanol per litre urine and where n = 10 determinations	
Detection limit:	0.05 mg isobutanol per litre urine	
Quantitation limit:	0.15 mg isobutanol per litre urine	

Acetone

Within-day precision:	Standard deviation (rel.)	s_w = 5.9% or 3.9%
	Prognostic range	u = 13.4% or 8.7%
	at a spiked concentration of 8.3 mg or 83.2 mg acetone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 7.0% or 4.3%
	Prognostic range	u = 16.5% or 10.1%
	at a spiked concentration of 8.3 mg or 83.2 mg acetone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 116% or 107%
	at a spiked concentration of 8.3 mg or 83.2 mg acetone per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg acetone per litre urine	
Quantitation limit:	0.03 mg acetone per litre urine	

2-Butanone (methyl ethyl ketone)

Within-day precision:	Standard deviation (rel.)	s_w = 8.1% or 2.4%
	Prognostic range	u = 18.4% or 5.3%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-butanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 3.6% or 2.5%
	Prognostic range	u = 8.5% or 5.8%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-butanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 105% or 107%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-butanone per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg 2-butanone per litre urine	
Quantitation limit:	0.03 mg 2-butanone per litre urine	

2-Pentanone

Within-day precision:	Standard deviation (rel.)	s_w = 4.0% or 3.7%
	Prognostic range	u = 9.1% or 8.3%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-pentanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 4.7% or 2.3%
	Prognostic range	u = 11.0% or 5.4%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-pentanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 103% or 110%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-pentanone per litre urine and where n = 10 determinations	
Detection limit:	0.02 mg 2-pentanone per litre urine	
Quantitation limit:	0.06 mg 2-pentanone per litre urine	

3-Pentanone

Within-day precision:	Standard deviation (rel.)	s_w = 3.3% or 4.4%
	Prognostic range	u = 7.6% or 10.0%
	at a spiked concentration of 0.2 mg or 2.5 mg 3-pentanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 1.9% or 3.2%
	Prognostic range	u = 4.5% or 7.5%
	at a spiked concentration of 0.2 mg or 2.5 mg 3-pentanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 95.5% or 111%
	at a spiked concentration of 0.2 mg or 2.5 mg 3-pentanone per litre urine and where n = 10 determinations	
Detection limit:	0.02 mg 3-pentanone per litre urine	
Quantitation limit:	0.06 mg 3-pentanone per litre urine	

3-Methyl-2-butanone (methyl isopropyl ketone)

Within-day precision:	Standard deviation (rel.)	s_w = 5.9% or 2.7%
	Prognostic range	u = 13.2% or 6.0%
	at a spiked concentration of 0.3 mg or 2.6 mg 3-methyl-2-butanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 6.4% or 2.6%
	Prognostic range	u = 15.2% or 6.2%
	at a spiked concentration of 0.3 mg or 2.6 mg 3-methyl-2-butanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 98.8% or 110%
	at a spiked concentration of 0.3 mg or 2.6 mg 3-methyl-2-butanone per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg 3-methyl-2-butanone per litre urine	
Quantitation limit:	0.03 mg 3-methyl-2-butanone per litre urine	

Cyclopentanone

Within-day precision:	Standard deviation (rel.)	s_w = 8.0% or 6.7%
	Prognostic range	u = 18.0% or 15.1%
	at a spiked concentration of 0.2 mg or 2.5 mg cyclopentanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 3.9% or 8.5%
	Prognostic range	u = 9.1% or 20.2%
	at a spiked concentration of 0.2 mg or 2.5 mg cyclopentanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 95.9% or 109%
	at a spiked concentration of 0.2 mg or 2.5 mg cyclopentanone per litre urine and where n = 10 determinations	
Detection limit:	0.05 mg cyclopentanone per litre urine	
Quantitation limit:	0.15 mg cyclopentanone per litre urine	

2-Hexanone

Within-day precision:	Standard deviation (rel.)	s_w = 3.8% or 4.5%
	Prognostic range	u = 8.5% or 10.1%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-hexanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 4.2% or 4.9%
	Prognostic range	u = 9.8% or 11.5%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-hexanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 85.0% or 112%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-hexanone per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg 2-hexanone per litre urine	
Quantitation limit:	0.03 mg 2-hexanone per litre urine	

3-Hexanone

Within-day precision:	Standard deviation (rel.)	s_w = 5.7% or 2.7%
	Prognostic range	u = 13.0% or 6.1%
	at a spiked concentration of 0.2 mg or 2.1 mg 3-hexanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 4.9% or 4.9%
	Prognostic range	u = 11.5% or 11.7%
	at a spiked concentration of 0.2 mg or 2.1 mg 3-hexanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 99.3% or 110%
	at a spiked concentration of 0.2 mg or 2.1 mg 3-hexanone per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg 3-hexanone per litre urine	
Quantitation limit:	0.03 mg 3-hexanone per litre urine	

3,3-Dimethyl-2-butanone (methyl-*tert*-butyl ketone)

Within-day precision:	Standard deviation (rel.)	s_w = 5.5% or 4.7%
	Prognostic range	u = 12.4% or 10.7%
	at a spiked concentration of 0.2 mg or 1.9 mg 3,3-dimethyl-2-butanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 1.9% or 5.1%
	Prognostic range	u = 4.5% or 12.1%
	at a spiked concentration of 0.2 mg or 1.9 mg 3,3-dimethyl-2-butanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 92.1% or 109%
	at a spiked concentration of 0.2 mg or 1.9 mg 3,3-dimethyl-2-butanone per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg 3,3-dimethyl-2-butanone per litre urine	
Quantitation limit:	0.03 mg 3,3-dimethyl-2-butanone per litre urine	

4-Methyl-2-pentanone (methyl isobutyl ketone)

Within-day precision:	Standard deviation (rel.)	s_w = 3.0% or 1.8%
	Prognostic range	u = 6.7% or 4.1%
	at a spiked concentration of 0.1 mg or 1.2 mg 4-methyl-2-pentanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 5.9% or 8.2%
	Prognostic range	u = 14.0% or 19.1%
	at a spiked concentration of 0.1 mg or 1.2 mg 4-methyl-2-pentanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 112% or 92.8%
	at a spiked concentration of 0.1 mg or 1.2 mg 4-methyl-2-pentanone per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg 4-methyl-2-pentanone per litre urine	
Quantitation limit:	0.03 mg 4-methyl-2-pentanone per litre urine	

Cyclohexanone

Within-day precision:	Standard deviation (rel.)	s_w = 10.0% or 4.2%
	Prognostic range	u = 22.7% or 9.4%
	at a spiked concentration of 0.3 mg or 2.6 mg cyclohexanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 5.1% or 4.4%
	Prognostic range	u = 12.1% or 10.5%
	at a spiked concentration of 0.3 mg or 2.6 mg cyclohexanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 103% or 99.7%
	at a spiked concentration of 0.3 mg or 2.6 mg cyclohexanone per litre urine and where n = 10 determinations	
Detection limit:	0.05 mg cyclohexanone per litre urine	
Quantitation limit:	0.15 mg cyclohexanone per litre urine	

2-Heptanone

Within-day precision:	Standard deviation (rel.)	s_w = 7.2% or 2.2%
	Prognostic range	u = 16.3% or 5.0%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-heptanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 3.4% or 9.5%
	Prognostic range	u = 8.1% or 22.4%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-heptanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 87.3% or 109%
	at a spiked concentration of 0.2 mg or 2.0 mg 2-heptanone per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg 2-heptanone per litre urine	
Quantitation limit:	0.03 mg 2-heptanone per litre urine	

3-Heptanone

Within-day precision:	Standard deviation (rel.)	s_w = 6.5% or 3.7%
	Prognostic range	u = 14.7% or 8.5%
	at a spiked concentration of 0.2 mg or 2.0 mg 3-heptanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 9.5% or 10.6%
	Prognostic range	u = 22.4% or 25.2%
	at a spiked concentration of 0.2 mg or 2.0 mg 3-heptanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 89.3% or 112%
	at a spiked concentration of 0.2 mg or 2.0 mg 3-heptanone per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg 3-heptanone per litre urine	
Quantitation limit:	0.03 mg 3-heptanone per litre urine	

4-Heptanone

Within-day precision:	Standard deviation (rel.)	s_w = 7.6% or 3.0%
	Prognostic range	u = 17.1% or 6.7%
	at a spiked concentration of 0.2 mg or 2.0 mg 4-heptanone per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 8.6% or 8.8%
	Prognostic range	u = 20.3% or 20.9%
	at a spiked concentration of 0.2 mg or 2.0 mg 4-heptanone per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 98.1% or 106%
	at a spiked concentration of 0.2 mg or 2.0 mg 4-heptanone per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg 4-heptanone per litre urine	
Quantitation limit:	0.03 mg 4-heptanone per litre urine	

Methyl-*tert*-butyl ether (MTBE)

Within-day precision:	Standard deviation (rel.)	s_w = 6.9% or 2.4%
	Prognostic range	u = 15.6% or 5.3%
	at a spiked concentration of 0.1 mg or 1.0 mg MTBE per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 9.2% or 5.3%
	Prognostic range	u = 21.8% or 12.6%
	at a spiked concentration of 0.1 mg or 1.0 mg MTBE per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 96.8% or 121%
	at a spiked concentration of 0.1 mg or 1.0 mg MTBE per litre urine and where n = 10 determinations	
Detection limit:	0.005 mg MTBE per litre urine	
Quantitation limit:	0.015 mg MTBE per litre urine	

Tetrahydrofuran (THF)

Within-day precision:	Standard deviation (rel.)	s_w = 7.4% or 2.0%
	Prognostic range	u = 16.7% or 4.5%
	at a spiked concentration of 0.2 mg or 2.2 mg THF per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 4.8% or 2.8%
	Prognostic range	u = 11.4% or 6.7%
	at a spiked concentration of 0.2 mg or 2.2 mg THF per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 90.6% or 113%
	at a spiked concentration of 0.2 mg or 2.2 mg THF per litre urine and where n = 10 determinations	
Detection limit:	0.01 mg THF per litre urine	
Quantitation limit:	0.03 mg THF per litre urine	

1,4-Dioxane

Within-day precision:	Standard deviation (rel.)	s_w = 7.0% or 4.6%
	Prognostic range	u = 15.9% or 10.4%
	at a spiked concentration of 0.2 mg or 1.7 mg 1,4-dioxane per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 6.6% or 4.6%
	Prognostic range	u = 15.7% or 10.8%
	at a spiked concentration of 0.2 mg or 1.7 mg 1,4-dioxane per litre urine and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 86.4% or 98.6%
	at a spiked concentration of 0.2 mg or 1.7 mg 1,4-dioxane per litre urine and where n = 10 determinations	
Detection limit:	0.10 mg 1,4-dioxane per litre urine	
Quantitation limit:	0.30 mg 1,4-dioxane per litre urine	

2 General information on the hazardous substances

Figure 1 shows the structural formulas of all the analytes that can be determined with this method.

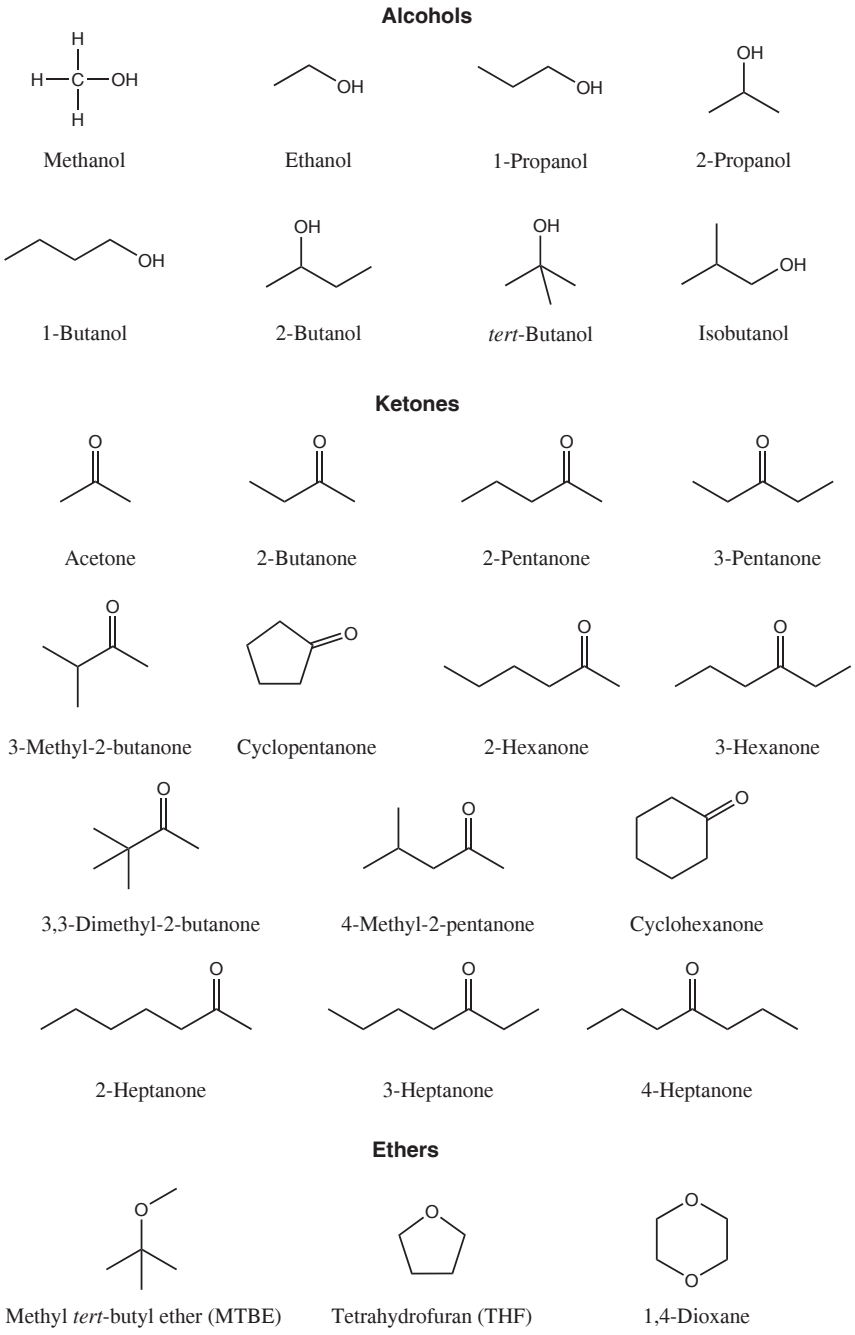


Fig. 1 Structural formulas of the analytes

Table 1 summarises the Commission's classifications and assessment values for the hazardous substances determined with this method, which correspond to the List of MAK and BAT Values of 2019 (DFG 2019). For each of the substances highlighted in bold in Table 1, there are biological assessment values available established by the

Commission, which can be verified using the method presented herein. These seven substances will therefore be discussed in more detail hereinafter.

Tab. 1 MAK values, classifications and assessment values by the Commission for the analytes of this method

Substance	MAK value	Assessment values in biological material
	Alcohols	
Methanol	MAK value: 100 ml/m³ (130 mg/m³); pregnancy risk group: C; danger from percutaneous absorption: H	BAT value: 15 mg/l (methanol in urine)[a), b)]
Ethanol	MAK value: 200 ml/m³ (380 mg/m³); pregnancy risk group: C; carcinogen category: 5; germ cell mutagen category: 5	–
2-Propanol	MAK value: 200 ml/m³ (500 mg/m³); pregnancy risk group: C	BAT value: 25 mg/l (acetone in urine)[a)] BAT value: 25 mg/l (acetone in blood)[a)]
1-Butanol	MAK value: 100 ml/m³ (310 mg/m³); pregnancy risk group: C	BAT value: 10 mg/g creatinine (1-butanol in urine)[a)] BAT value: 2 mg/g creatinine (1-butanol in urine)[c)]
2-Butanol	–[e)]	–
tert-Butanol	MAK value: 20 ml/m³ (62 mg/m³); pregnancy risk group: C	–
Isobutanol	MAK value: 100 ml/m³ (310 mg/m³); pregnancy risk group: C	–
	Ketones	
Acetone	MAK value: 500 ml/m³ (1200 mg/m³); pregnancy risk group: B[d)]	BAT value: 80 mg/l (acetone in urine)[a)]
2-Butanone	MAK value: 200 ml/m³ (600 mg/m³); pregnancy risk group: C; danger from percutaneous absorption: H	BAT value: 2 mg/l (2-butanone in urine)[a)]
2-Pentanone	–[e)]	–
2-Hexanone	MAK value: 5 ml/m³ (21 mg/m³); danger from percutaneous absorption: H	BAT value: 5 mg/l (2,5-hexanedione plus 4,5-dihydroxy-2-hexanone (after hydrolysis) in urine)[a), b)]
4-Methyl-2-pentanone	MAK value: 20 ml/m³ (83 mg/m³); pregnancy risk group: C; danger from percutaneous absorption: H	BAT value: 0.7 mg/l (4-methyl-2-pentanone in urine)[a)]
Cyclohexanone	MAK value: –; danger from percutaneous absorption: H; carcinogen category: 3B	EKA: 1,2-cyclohexanediol in urine[b)] EKA: cyclohexanol in urine[a)]
3-Heptanone	MAK value: 10 ml/m³ (47 mg/m³); pregnancy risk group: D	–
	Ethers	
MTBE	MAK value: 50 ml/m³ (180 mg/m³); pregnancy risk group: C; carcinogen category: 3B	–[e)]
THF	MAK value: 50 ml/m³ (150 mg/m³); pregnancy risk group: C; danger from percutaneous absorption: H; carcinogen category: 4	BAT value: 2 mg/l (THF in urine)[a)]
1,4-Dioxane	MAK value: 20 ml/m³ (73 mg/m³); pregnancy risk group: C; danger from percutaneous absorption: H; carcinogen category: 4	BAT value: 200 mg/g creatinine (2-hydroxyethoxy acetic acid in urine)[a)]

[a)] Sampling time: end of exposure or end of shift
[b)] Sampling time: for long-term exposure: at the end of the shift after several shifts
[c)] Sampling time: at the beginning of the next shift
[d)] indication of prerequisite for Group C, see MAK value documentation
[e)] MAK value or assessment value could not be derived, but documentation is available

Methanol Methanol is the simplest member of the group of alcohols. Under standard conditions, it is a clear, colourless and highly volatile liquid. Methanol is widely used as a solvent and also serves as a starting material in

the chemical industry for the production of a large number of other chemicals, such as formaldehyde, formic acid and acetic acid (Falbe and Regitz 1991). For methanol, the Commission established a MAK value of 100 ml/m³ and it was designated with an "H" (danger from percutaneous absorption). For details on the toxicological evaluation of methanol, please refer to the relevant MAK Value Documentations (Greim 2001; Hartwig and MAK Commission 2019 a). Methanol is readily absorbed by inhalation, ingestion and following dermal exposure and it is rapidly distributed in the organism, regardless of the route of exposure. It may be exhaled or excreted via urine and faeces, as well unchanged as in form of metabolites (Greim 2001).

In urine, methanol is mainly excreted unchanged and in the form of the metabolite formate. Due to an elevated endogenous formate formation, the determination of formate in urine is not suitable as a biomarker of exposure to methanol. As methanol is also formed endogenously to a small extent, background levels of methanol in blood and urine are also found in individuals not occupationally exposed to methanol. The mean background level in the urine of the general population ranges between 0.7 and 2.1 mg methanol/l. The elimination half-life of methanol in urine is reported to be 1.5 to 2 hours. Since the half-life of methanol in blood is significantly shorter than that in urine, human biomonitoring in urine is to be preferred. Urine sampling should be performed immediately after the end of exposure or the end of shift. In correlation with the MAK value, a BAT value of 15 mg/l urine was derived for methanol (Kreis et al. 2019).

2-Propanol 2-Propanol is the simplest secondary alcohol. Under standard conditions, it is a clear, colourless and highly volatile liquid. It is used as a solvent and diluent as well as a starting material in the synthesis of acetone and other chemicals (IARC 1999). A MAK value of 200 ml/m³ was derived for 2-propanol. For details on the toxicological evaluation of 2-propanol, please refer to the relevant MAK Value Documentations (Hartwig 2013 a; Hartwig and MAK Commission 2019 b) and an IARC monograph (IARC 1999). 2-Propanol is readily absorbed by inhalation and ingestion, whereas percutaneous absorption is rather low. About 85% of absorbed 2-propanol is oxidised by hepatic alcohol dehydrogenase to acetone, which is partly further metabolised to formic acid, carbon dioxide and water (Schaller and Triebig 1994 a). 2-Propanol is either eliminated via exhaled air or excreted in urine, both unchanged and in the form of its main metabolite acetone. The elimination half-life of 2-propanol is reported to be 2.5–6.4 hours, while the elimination half-life of acetone formed from 2-propanol is significantly longer at 11.0–22.4 hours (Hartwig and MAK Commission 2019 b; IARC 1999). The blood and urinary levels of acetone have proved to be suitable biomarkers of occupational exposure to 2-propanol. The Commission therefore derived a BAT value of 25 mg acetone/l blood or urine for 2-propanol in correlation with the MAK value. Sampling should be performed at the end of exposure or end of shift (Schaller 2011; Schaller and Triebig 1994 a). Acetone is formed endogenously and is therefore also found in the urine of non-occupationally exposed individuals. The urinary excretion levels of acetone are usually below 3 mg/l. Increased physiological background levels of 30–40 mg/l are observed for people with poorly controlled diabetes and in fasting persons (Schaller and Triebig 1998). When assessing these values, one should always consider a potential exposure to acetone itself.

1-Butanol 1-Butanol is a primary alcohol. Under standard conditions, it is a colourless, flammable liquid with a characteristic odour. It is used as a solvent and extracting agent as well as a starting material or intermediate in the synthesis of various ethers and esters (Falbe and Regitz 1991; Koss 2004). For 1-butanol, a MAK value of 100 ml/m³ was derived based on its irritating effect upon the eyes. For details on the toxicological evaluation of 1-butanol, please refer to the relevant MAK Value Documentations (Greim 2003; Hartwig and MAK Commission 2016 b). 1-Butanol is rapidly absorbed through the lungs and by the gastrointestinal tract. There is no adequate data concerning percutaneous absorption of 1-butanol in humans (Greim 2003; Hartwig and MAK Commission 2016 b). After inhalation exposure to 1-butanol, absorption rates in humans range between 40 and 60%. Animal studies have shown that 1-butanol is metabolised to 1-butanal and may be further oxidised to butyric acid. Subsequently, it is degraded to its main metabolite carbon dioxide. Accordingly, rats exposed to 1-butanol excreted 83% of the dose via exhaled air and only about 4% via urine. In urine, 1-butanol is excreted mainly as conjugates (sulfate, glucuronide) (Greim 2003; Koss 2004). Taking into account the feasibility and the available data, the determination of 1-butanol in urine is recommended as a suitable biomarker of occupational exposure to 1-butanol. For this

reason, the Commission derived a BAT value of 2 mg 1-butanol/l urine (sampling at the beginning of the next shift) or 10 mg 1-butanol/l urine (sampling at the end of exposure or end of shift) for 1-butanol in correlation with the MAK value (Lewalter et al. 2010; Weistenhöfer et al. 2017). The background level of 1-butanol in the urine of the general population is relatively low at levels ranging between 20 and 47 µg/l (Lewalter et al. 2010).

Acetone Acetone is the simplest, but with respect to its production volume the most important member of the group of aliphatic ketones. Under standard conditions, it is a clear, colourless liquid with an aromatic odour. It is widely used as a solvent and extracting agent as well as a starting material in the synthesis of numerous compounds (Falbe and Regitz 1991). Based on findings on mood and irritation, a MAK value of 500 ml/m^3 was established for acetone. In addition, acetone was classified in Pregnancy Risk Group B. For details on the toxicological evaluation of acetone and indications of prerequisites for classification in Pregnancy Risk Group C, please refer to the relevant MAK Value Documentations (Greim 1996 a; Hartwig and MAK Commission 2016 a). Acetone is readily absorbed by inhalation with an absorption rate of about 45%. Percutaneous absorption is estimated to be rather low. Metabolism of acetone is dose-dependent, with unchanged acetone being increasingly exhaled or excreted in urine with increasing exposure levels. Acetone is metabolised by cytochrome P450-dependent oxidation to methylglyoxal or 1,2-propanediol and subsequently degraded to carbon dioxide and water. Acetone is primarily eliminated via exhaled air. Approximately 1–15% of the absorbed dose are excreted in urine (Greim 1996 a; Schaller and Triebig 1996). The half-life of acetone in human plasma is reported to be 3.5–4 hours (Greim 1996 b). Acetone is formed endogenously and is therefore also found in the urine of non-occupationally exposed individuals. The physiological urinary excretion levels of acetone are usually below 3 mg/l. Increased physiological background levels of 30–40 mg/l are observed for people with poorly controlled diabetes and in fasting persons. In addition, it should be considered that an exposure to 2-propanol also leads to an increased acetone excretion. In correlation with the MAK value, the Commission derived a BAT value of 80 mg acetone/l urine, with sampling being performed immediately after the end of exposure or end of shift (Schaller and Triebig 1998).

2-Butanone (methyl ethyl ketone) 2-Butanone, also generally known as methyl ethyl ketone, is one of the most important industrially used ketones besides acetone. Under standard conditions, it is a clear, colourless liquid with an aromatic odour. Like acetone, 2-butanone is primarily used as a solvent and in dewaxing of lubricating oils (Falbe and Regitz 1991). For 2-butanone, the Commission established a MAK value of 200 ml/m^3 based on its irritant effect upon the eyes, nose and throat and designated it with an "H" (danger from percutaneous absorption). For details on the toxicological evaluation of 2-butanone, please refer to the relevant MAK Value Documentations (Greim 1999 a; Henschler 1977). 2-Butanone is rapidly absorbed following inhalation, dermal exposure or ingestion. The absorption rate in humans after inhalation exposure is between 53–70% (Greim 1999 a). Approximately 25% of the absorbed 2-butanone is exhaled unchanged. The remaining share is metabolised and eliminated via the lungs or the kidneys. Only about 0.1% is excreted in urine as unchanged 2-butanol. 3-Hydroxy-2-butanone, 2-butanol and 2,3-butanediol were identified as urinary metabolites of 2-butanone. In animal experiments, the elimination half-life of 2-butanone in blood was found to be 4.5 hours (Angerer 1995; Greim 1999 a). Based on the known toxicokinetic data for 2-butanone, it can be assumed that it does not accumulate in the body in case of occupational exposure. On the basis of the data available and the fact that studies found a strong correlation between the concentration of 2-butanone in the workplace air and the urinary excretion of 2-butanone, the Commission derived a BAT value of 2 mg 2-butanone/l urine in correlation with the MAK value for 2-butanone. Sampling should be performed at the end of exposure or end of shift (Angerer 1995; Nasterlack 2014).

4-Methyl-2-pentanone (methyl isobutyl ketone) Under standard conditions, 4-methyl-2-pentanone is a clear, colourless liquid with a sweetish odour. It is also known as methyl isobutyl ketone. 4-Methyl-2-pentanone is widely used as a solvent and extracting agent in various industrial processes. It also occurs as an intermediate of some syntheses in the industrial polymer production (IARC 2013; Schaller and Triebig 1994 b). Based on the irritating effect of 4-methyl-2-pentanone upon mucous membranes, the Commission derived a MAK value of 20 ml/m^3 and designated 4-methyl-2-pentanone with an "H" (danger from percutaneous absorption). For details on the toxicological evaluation of 4-methyl-2-pentanone, please refer to the relevant MAK Value Documentations (Greim 1999 b,

2000) and an IARC monograph (IARC 2013). Additionally, the IARC (2013) classified 4-methyl-2-pentanone as a carcinogen of group 2B (possibly carcinogenic to humans). The Commission, however, did not classify 4-methyl-2-pentanone as a carcinogenic substance. The main route of occupational exposure to 4-methyl-2-pentanone is by inhalation, with pulmonary absorption in humans being about 60% (Greim 1999 b; IARC 2013). 4-Methyl-2-pentanone is eliminated quite rapidly following two-phase elimination kinetics with half-lives in the blood of 12 minutes and 70 minutes, respectively (Greim 1999 b). The most important route of elimination of 4-methyl-2-pentanone is exhalation via the lungs. Urinary excretion is a secondary route of elimination, with only 0.04% being excreted unchanged in urine (Greim 1999 b; IARC 2013; Schaller and Triebig 1994 b). The metabolism of 4-methyl-2-pentanone has so far only been investigated in animals, with 4-methyl-2-pentanol and 4-hydroxy-4-methyl-2-pentanone being the main metabolites detected in blood. So far, these metabolites have not been detected in the urine of occupationally exposed individuals (IARC 2013; Schaller and Triebig 1994 b). Based on the known toxicokinetic data for 4-methyl-2-pentanone, it can be assumed that it does not accumulate in the body in case of occupational exposure. It is excreted very rapidly after the end of exposure, with the highest concentration of 4-methyl-2-pentanone being found in urine immediately at the end of exposure. Various studies show a linear correlation between the 4-methyl-2-pentanone exposure dose and the 4-methyl-2-pentanone level in urine. Therefore, the Commission derived a BAT value of 0.7 mg 4-methyl-2-pentanone/l urine in correlation with the MAK value. Sampling should be performed immediately at the end of exposure or end of shift (Nasterlack et al. 2017; Schaller and Triebig 1994 b).

Tetrahydrofuran (THF) THF belongs to the class of cyclic ethers. Under standard conditions, it is a clear, colourless and highly volatile liquid with an ether-like odour. THF is used for a variety of applications. It is used as a solvent and it is also an important intermediate in the polyamide, polyester and polyurethane production (IARC 2019). Due to morphological changes in the nasal mucosa, the Commission derived a MAK value of 50 ml/m³ for THF and designated it with an "H" (danger from percutaneous absorption). In addition, THF was classified by the Commission as a Category 4 carcinogen. The IARC (2019) also classifies THF as possibly carcinogenic to humans (Group 2B). For details on the toxicological evaluation of THF, please refer to the relevant MAK Value Documentation (Hartwig 2013 b) and an IARC monograph (IARC 2019). By inhalation, THF is absorbed rapidly to a large extent. Studies show that it is also readily absorbed through the skin (Hartwig 2013 b; IARC 2019). THF is rapidly metabolised in the organism, mainly to carbon dioxide, which is subsequently exhaled. Another metabolite described in animal experiments is gamma-4-hydroxy butyric acid (IARC 2019). THF has a low potential for bioaccumulation (ECHA 2020; IARC 2019). Animal studies on rats have shown that a large proportion of a radioactively labelled THF dose is exhaled as carbon dioxide. At about 2–4%, urinary excretion constitutes only a secondary route of elimination. In general, most of a THF dose is eliminated within the first 24 hours (ECHA 2020). Background levels of THF in the blood or urine of individuals not occupationally exposed to THF have not been described so far (Lewalter 1995). Studies show a good correlation between the THF level in urine and external THF exposure. The correlation with THF in blood, however, is significantly worse. Therefore, the Commission derived a BAT value of 2 mg THF/l urine in correlation with the MAK value. Sampling should be performed at the end of exposure or end of shift (Lewalter 1995; Lewalter and Leng 2005).

3 General principles

The analytical method described hereinafter permits the simultaneous determination of various alcohols, ketones and ethers in urine. Methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, *tert*-butanol and isobutanol are determined in the group of alcohols. In the group of ketones, acetone, 2-butanone (methyl ethyl ketone), 2-pentanone, 3-pentanone, 3-methyl-2-butanone, cyclopentanone, 2-hexanone, 3-hexanone, 3,3-dimethyl-2-butanone (methyl-*tert*-butyl ketone), 4-methyl-2-pentanone (methyl isobutyl ketone), cyclohexanone, 2-heptanone, 3-heptanone and 4-heptanone are determined. In addition, this method can be used for the determination of the ethers methyl-*tert*-butyl ether (MTBE), tetrahydrofuran (THF) and 1,4-dioxane. For determination, the internal standards (ISTD) are added into gas-tight headspace vials containing the urine samples. The solutions are heated

to 60 °C in the autosampler and then an aliquot of the headspace phase is transferred to the gas chromatograph and analysed by mass spectrometry. Calibration standards are prepared in water and processed in the same way as the samples to be analysed.

4 Equipment, chemicals and solutions

4.1 Equipment

- Gas chromatograph with mass spectrometer (e.g. Agilent 5890 A with Agilent 5975 C, Agilent Technologies Deutschland GmbH, Waldbronn, Germany)
- Headspace autosampler (e.g. PerkinElmer Inc., Rodgau, Germany)
- Capillary gas chromatography column: stationary phase: 6% cyanopropyl-phenyl-methylpolysiloxane, length: 60 m; inner diameter: 0.32 mm; film thickness: 1.8 µm (e.g. VF-624 ms by Agilent Technologies Deutschland GmbH, Waldbronn, Germany, No. CP9105)
- 20 ml headspace vials (e.g. Agilent Technologies Deutschland GmbH, Waldbronn, Germany, No. 5183-4474)
- Aluminium crimp caps with Teflon-coated butyl septa (e.g. Agilent Technologies Deutschland GmbH, Waldbronn, No. 5183-4479)
- Microliter syringe, 25 µl (e.g. Hamilton Medical AG, Bonaduz, Switzerland, No. 80439)
- Various pipettes (e.g. Eppendorf AG, Hamburg, Germany)
- Various volumetric flasks (e.g. VWR International GmbH, Darmstadt, Germany)
- Roller mixer (e.g. VWR International GmbH, Darmstadt, Germany)
- Analytical balance (e.g. Sartorius AG, Göttingen, Germany)

4.2 Chemicals

Unless otherwise specified, all chemicals must be at least p.a. grade.

- Methanol (e.g. Merck KGaA, Darmstadt, Germany, No. 100837)
- Ethanol, absolute (e.g. Merck KGaA, Darmstadt, Germany, No. 100983)
- 1-Propanol (e.g. Merck KGaA, Darmstadt, Germany, No. 100997)
- 2-Propanol (e.g. Merck KGaA, Darmstadt, Germany, No. 109634)
- 1-Butanol (e.g. Merck KGaA, Darmstadt, Germany, No. 101990)
- 2-Butanol (e.g. Merck KGaA, Darmstadt, Germany, No. 109630)
- *tert*-Butanol (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 50621)
- Isobutanol (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 294829)
- Acetone (e.g. Merck KGaA, Darmstadt, Germany, No. 100014)
- 2-Butanone (methyl ethyl ketone) (e.g. Merck KGaA, Darmstadt, Germany, No. 109708)
- 2-Pentanone (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 46211)

- 3-Pentanone (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 40129)
- 3-Methyl-2-butanone (e.g. TCI Deutschland GmbH, Eschborn, Germany, No. M0173)
- Cyclopentanone (e.g. Merck KGaA, Darmstadt, Germany, No. 802670)
- 2-Hexanone (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 47733-U)
- 3-Hexanone (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 103020)
- 3,3-Dimethyl-2-butanone (methyl-*tert*-butyl ketone) (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. P45605)
- 4-Methyl-2-pentanone (methyl isobutyl ketone) (e.g. Merck KGaA, Darmstadt, Germany, No. 106146)
- Cyclohexanone (e.g. Merck KGaA, Darmstadt, Germany, No. 102888)
- 2-Heptanone (e.g. Merck KGaA, Darmstadt, Germany, No. 818711)
- 3-Heptanone (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. H6511)
- 4-Heptanone (e.g. Merck KGaA, Darmstadt, Germany, No. 803505)
- MTBE (e.g. Merck KGaA, Darmstadt, Germany, No. 101995)
- THF (e.g. Merck KGaA, Darmstadt, Germany, No. 109731)
- 1,4-Dioxane (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 296309)
- D_8-2-Propanol (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 175897)
- D_5-4-Methyl-2-pentanone (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 487724)
- Ultrapure water (e.g. Merck KGaA, Darmstadt, Germany, No. 115333)
- Helium 5.0 (e. g. Linde AG, Pullach, Germany)

4.3 Internal standards (ISTD)

- ISTD spiking solution (210 mg/l D_5-4-methyl-2-pentanone, 890 mg/l D_8-2-propanol)

  50 ml ultrapure water are placed into a 100 ml volumetric flask and 25 µl D_5-4-methyl-2-pentanone as well as 100 µl D_8-2-propanol are added using a pipette. The flask is then made up to the mark with ultrapure water.

The ISTD spiking solution is stable for at least one year when stored in the refrigerator at 4 °C.

4.4 Calibration standards

- Stock solution 1

  750 mg each of methanol and ethanol, 1125 mg acetone, 125 mg each of 1-propanol, 2-propanol, isobutanol, *tert*-butanol and 2-butanol, 250 mg 1-butanol, 62.5 mg each of 3-methyl-2-butanone, 2-butanone, 3,3-dimethyl-2-butanone, 4-methyl-2-pentanone, 2-pentanone, 3-pentanone, cyclopentanone, 2-hexanone, 3-hexanone, cyclohexanone, 2-heptanone, 3-heptanone, 4-heptanone and THF as well as 25 mg each of MTBE and 1,4-dioxane are weighed exactly into a 10 ml volumetric flask, which is then made up to the mark with ultrapure water. Stock solution 1 contains a concentration of 75 g/l each of methanol and ethanol, 112.5 g/l

acetone, 12.5 g/l each of 1-propanol, 2-propanol, isobutanol, *tert*-butanol and 2-butanol, 25 g/l 1-butanol, 6.25 g/l each of 3-methyl-2-butanone, 2-butanone, 3,3-dimethyl-2-butanone, 4-methyl-2-pentanone, 2-pentanone, 3-pentanone, cyclopentanone, 2-hexanone, 3-hexanone, cyclohexanone, 2-heptanone, 3-heptanone, 4-heptanone and THF as well as 2.5 g/l each of MTBE and 1,4-dioxane.

- Stock solution 2

  2 ml of stock solution 1 are pipetted into a 25 ml volumetric flask, which is made up to the mark with ultrapure water. Stock solution 2 contains a concentration of 6 g/l each of methanol and ethanol, 9 g/l acetone, 1 g/l each of 1-propanol, 2-propanol, isobutanol, *tert*-butanol and 2-butanol, 2 g/l 1-butanol, 0.5 g/l each of 3-methyl-2-butanone, 2-butanone, 3,3-dimethyl-2-butanone, 4-methyl-2-pentanone, 2-pentanone, 3-pentanone, cyclopentanone, 2-hexanone, 3-hexanone, cyclohexanone, 2-heptanone, 3-heptanone, 4-heptanone and THF as well as 0.2 g/l each of MTBE and 1,4-dioxane.

- Spiking solution 1

  1 ml of stock solution 2 is pipetted into a 50 ml volumetric flask, which is made up to the mark with ultrapure water.

- Spiking solution 2

  0.1 ml of stock solution 2 are pipetted into a 50 ml volumetric flask, which is made up to the mark with ultrapure water.

The stock solutions and spiking solutions are stable for at least one year when stored in the refrigerator at 4 ℃.

The calibration standards are prepared in water, as the slopes of the calibration curves of the individual analytes are very similar in water and urine and thus lead to similar analytical results (see Sections 8 and 9). The corresponding volumes of water and of the spiking solutions according to Table 2 are placed into 20 ml headspace vials, which are then sealed with aluminium crimp caps with Teflon-coated butyl septa. Then, 10 µl of the ISTD spiking solution are added through the septum with a 25 µl microliter syringe. The calibration standards prepared in this way are mixed on a roller mixer for 1 hour and can then directly be used for analysis.

Tab. 2 Pipetting scheme for the preparation of calibration standards used to determine alcohols, ketones and ethers in urine

Calibration standard	Spiking solution 1 [µl]	Spiking solution 2 [µl]	Water [µl]
0	–	–	2000
1	–	50	1950
2	–	100	1900
3	–	150	1850
4	–	200	1800
5	–	300	1700
6	50	–	1950
7	100	–	1900
8	150	–	1850
9	200	–	1800
10	300	–	1700
11	500	–	1500
12	1000	–	1000

The resulting analyte levels in the calibration standards are presented in Table 3.

Tab. 3 Concentration levels of the analytes in the calibration standards (cf. Table 2)

Calibration standard	Methanol, ethanol [mg/l]	Acetone [mg/l]	1-Butanol [mg/l]	1-Propanol, 2-propanol, isobutanol, *tert*-butanol, 2-butanol [mg/l]	MTBE, 1,4-dioxane [mg/l]	Further analytes [mg/l]
0	–	–	–	–	–	–
1	0.3	0.45	0.1	0.05	0.01	0.025
2	0.6	0.90	0.2	0.10	0.02	0.05
3	0.9	1.35	0.3	0.15	0.03	0.075
4	1.2	1.8	0.4	0.20	0.04	0.10
5	1.8	2.7	0.6	0.30	0.06	0.15
6	3.0	4.5	1.0	0.50	0.10	0.25
7	6.0	9.0	2.0	1.0	0.20	0.50
8	9.0	13.5	3.0	1.5	0.30	0.75
9	12	18	4.0	2.0	0.40	1.0
10	18	27	6.0	3.0	0.60	1.5
11	30	45	10	5.0	1.0	2.5
12	60	90	20	10	2.0	5.0

5 Specimen collection and sample preparation

The samples are stored at −20 ℃ until analysis. Prior to analysis, the samples are thawed at room temperature and mixed thoroughly. The work up of the urine samples correspond to the preparation of the calibration standards. To this end, 2 ml of the urine sample are placed into a 20 ml headspace vial, which is sealed with a aluminium crimp cap with Teflon-coated butyl septum. 10 µl of the ISTD spiking solution are then added through the septum. The samples prepared in this way are mixed on a roller mixer for 1 hour and can then be used directly for analysis.

6 Operational parameters

Analysis was performed using a gas chromatograph coupled with a headspace autosampler, a mass selective detector and a data processing system.

6.1 Headspace autosampler

Equilibration time:	60 min at 60 ℃
Transfer line to the GC:	120 ℃
Needle temperature:	70 ℃
Pressure build-up:	18 psi for 0.5 min
Injection time:	0.08 min

6.2 Gas chromatography

Capillary column:	Stationary phase:	VF-624 ms (6%-cyanopropyl-phenyl-methyl-polysiloxane)
	Length:	60 m
	Inner diameter:	0.32 mm
	Film thickness:	1.8 µm
Temperature:	Headspace oven:	60 °C (60 min)
	Column:	Initial temperature 45 °C, 10 min hold time, increase at a rate of 5 °C/min to 110 °C, 5 min hold time, then increase at a rate of 10 °C/min to 220 °C
	Injector:	130 °C
	Transfer line:	280 °C
Carrier gas:	Helium 5.0	
Flow rate:	1.2 ml/min	
Injection:	Split 1 : 5	

6.3 Mass spectrometry

Ionisation mode:	Electron ionisation (EI)
Ionisation energy:	70 eV
Source temperature:	230 °C
Quadrupole temperature:	150 °C
Dwell time:	50 ms
Detection mode:	Single Ion Monitoring (SIM)

All parameters are instrument-specific and must be adjusted individually by the user. The parameters specified above are therefore intended as a rough guide only. All other parameters have to be optimised in accordance with the manufacturer's specifications.

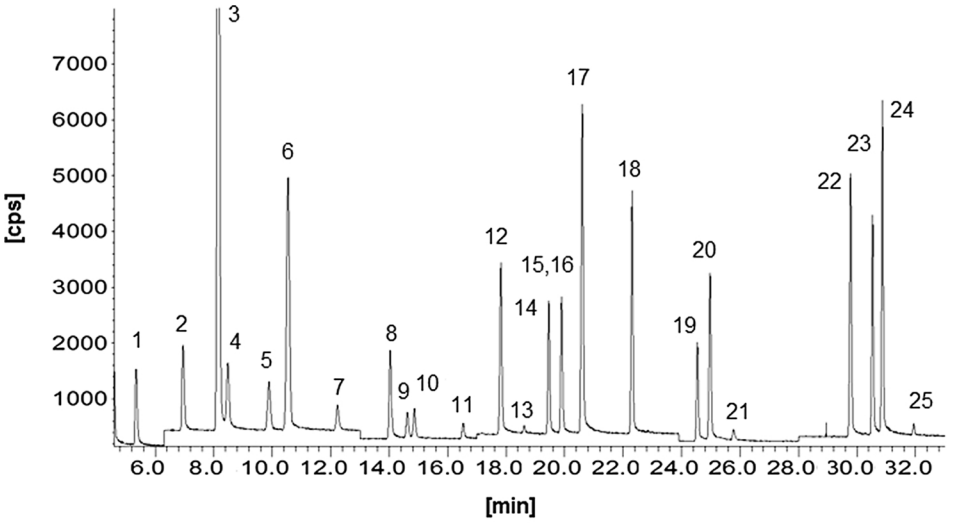
7 Analytical determination

For analytical determination of the urine samples prepared as described in Section 5, an aliquot of the sample's headspace phase is injected into the GC-MS system after heating the samples at 60 °C for 1 hour in the headspace oven. Identification of the analytes is based on retention times and characteristic ion traces. The temporal profiles of the ion traces shown in Table 4 are recorded in SIM mode. A quality control sample and a reagent blank consisting of ultrapure water are included in each analytical run.

Tab. 4 Retention times and detected ion traces of the analytes and ISTD

Analyte	Retention time [min]	Ion trace [*m/z*]	
		Quantifier	Qualifier
Methanol	5.3	31	29
Ethanol	7.0	31	45
Acetone	8.2	43	58
2-Propanol	8.5	45	43
tert-Butanol	9.9	59	41
MTBE	10.6	73	57
1-Propanol	12.2	31	59
2-Butanone	14.0	43	72
2-Butanol	14.6	45	59
THF	14.9	42	72
Isobutanol	16.5	43	74
3-Methyl-2-butanone	17.3	43	86
1-Butanol	18.6	56	43
2-Pentanone	19.5	43	86
3-Pentanone	19.9	57	86
1,4-Dioxane	19.9	88	–
3,3-Dimethyl-2-butanone	20.6	57	100
4-Methyl-2-pentanone	22.3	43	58
3-Hexanone	24.5	43	100
2-Hexanone	25.0	43	58
Cyclopentanone	25.8	55	84
4-Heptanone	29.8	71	43
3-Heptanone	30.5	57	114
2-Heptanone	30.9	43	58
Cyclohexanone	31.9	55	98
D_8-2-Propanol	8.5	49	–
D_5-4-Methyl-2-pentanone	22.3	63	–

The retention times given are intended as a rough guide only. Users must ensure proper separation performance of the capillary column used influencing the resulting retention behaviour of the analytes. Figure 2 shows a chromatogram of a urine sample spiked with the standard solutions.



1	Methanol	10	THF	18	4-Methyl-2-pentanone
2	Ethanol	11	Isobutanol	19	3-Hexanone
3	Acetone	12	3-Methyl-2-butanone	20	2-Hexanone
4	2-Propanol	13	1-Butanol	21	Cyclopentanone
5	tert-Butanol	14	2-Pentanone	22	4-Heptanone
6	MTBE	15	3-Pentanone	23	3-Heptanone
7	1-Propanol	16	1,4-Dioxane	24	2-Heptanone
8	2-Butanone	17	3,3-Dimethyl-2-butanone	25	Cyclohexanone
9	2-Butanol				

Fig. 2 GC-MS chromatogram of a urine sample spiked with standard solutions

8 Calibration

The calibration standards (see Section 4.4) are analysed in the same way as the urine samples according to Section 6. Usually, the calibration curves are automatically plotted by the instrument software using quadratic regression. However, since this method may yield unacceptable validation data in the low concentration range for some analytes, depending on the instrument used, it is advisable to generate a linear calibration curve in the low concentration range for selected analytes (see Table 5) by plotting the quotients of the peak areas of the analytes and the internal standards against their respective spiked concentrations. For the alcoholic analytes, the internal standard D_8-2-propanol is used and for the ethers and ketones detected by this method, the internal standard D_5-4-methyl-2-pentanone is used. Figure 3 shows the calibration graphs of two analytes.

Tab. 5 Linear calibration ranges for selected analytes in urine

Analyte	Linear calibration range [mg/l]
2-Butanone	0.025–1.00
2-Pentanone	0.025–0.50
3-Pentanone	0.025–0.50
Cyclopentanone	0.025–1.50
2-Hexanone	0.025–0.50
3-Hexanone	0.025–0.50

Tab. 5 *(continued)*

Analyte	Linear calibration range [mg/l]
3,3-Dimethyl-2-butanone	0.025–0.50
4-Methyl-2-pentanone	0.025–1.00
2-Heptanone	0.025–0.25
3-Heptanone	0.025–0.50
4-Heptanone	0.025–0.80
THF	0.025–0.80
1,4-Dioxane	0.010–0.20

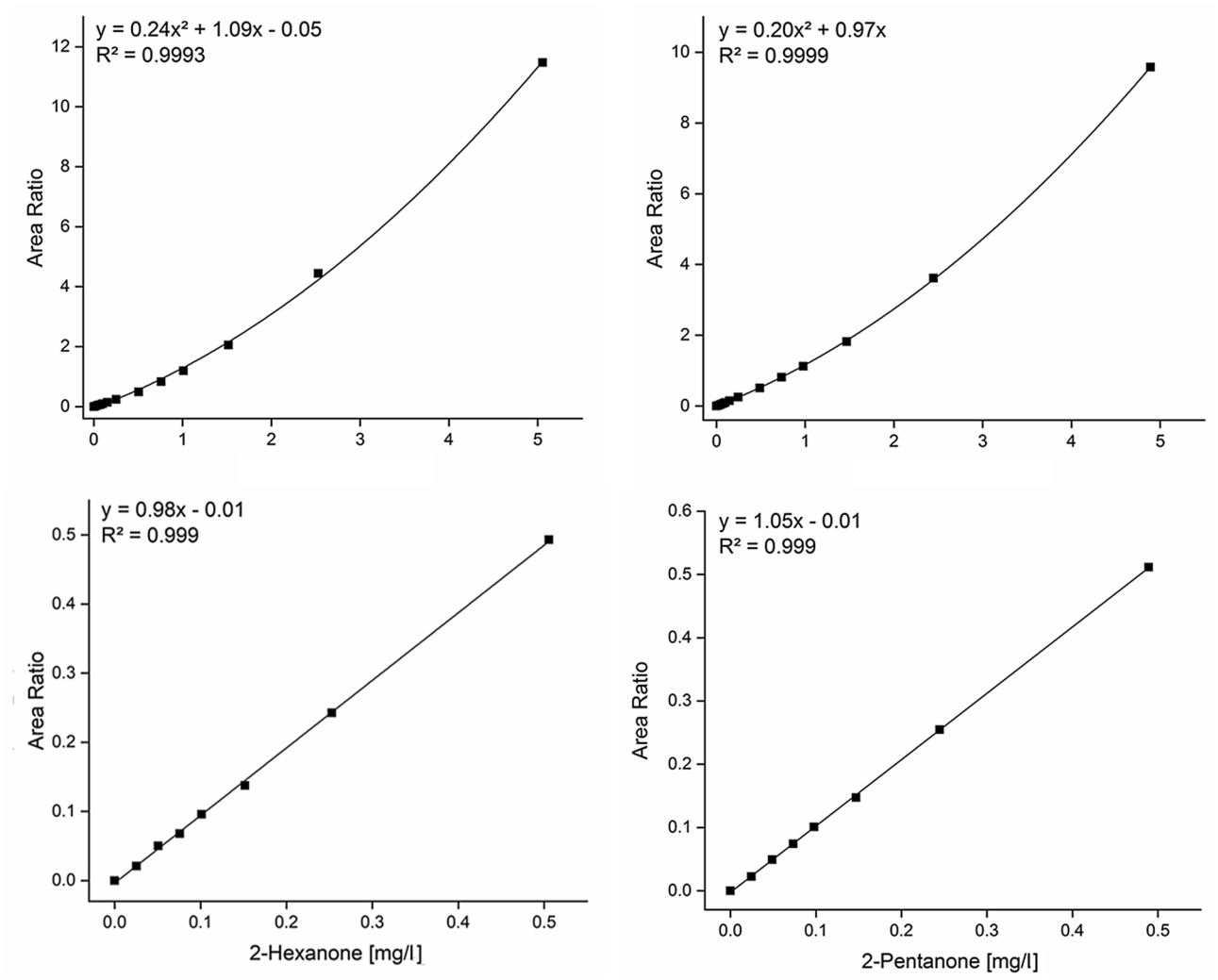


Fig. 3 Example of calibration curves for the analytes 2-hexanone and 2□pentanone. Top row: quadratic regression for the entire calibration range; bottom row: linear regression in the lower calibration range

9 Calculation of the analytical results

The analyte concentrations of the urine samples are calculated and provided by the instrument software using quadratic or linear regression (Section 8), leading to analyte levels in mg/l. Any reagent blank values have to be subtracted from the analytical results.

10 Standardisation and quality control

Quality control of the analytical results is carried out as stipulated in the guidelines of the Bundesärztekammer (German Medical Association) and in a general chapter of the MAK Collection for Occupational Health and Safety (Bader et al. 2010; Bundesärztekammer 2014). To check precision, quality control samples with known and constant analyte concentrations are analysed within each analytical run. As material for quality control is not commercially available, it must be prepared in the laboratory. To this end, spiking solutions of the analytes at two concentration levels are added to pooled urine so that the concentration of the control material is within the relevant concentration range. The quality control material is aliquoted to 2 ml into 20 ml headspace-vials and frozen at −20 °C. The nominal value and the tolerance ranges (mean value ± two standard deviations) of the quality control material are determined in a pre-analytical period (Bader et al. 2010).

11 Evaluation of the method

The reliability of the method was verified by comprehensive validation as well as by implementation and validation of the method in two independent laboratories.

11.1 Precision

To determine within-day precision, urine samples were spiked with the analytes, processed and analysed. Tenfold determination of these urine samples yielded the within-day precision data presented in Table 6.

Tab. 6 Within-day precision for the determination of the analytes in urine (n = 10)

Analyte	Spiked concentration [mg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
		Alcohols	
Methanol	2.9	6.2	14.0
	29.4	2.3	5.2
Ethanol	3.0	4.6	10.4
	30.2	2.2	5.0
1-Propanol	0.5	9.7	21.9
	5.3	2.4	5.4
2-Propanol	0.5	2.5	5.7
	5.4	1.8	4.0
1-Butanol	1.0	8.1	18.4
	9.9	2.2	5.0
2-Butanol	0.5	6.2	14.0
	5.3	1.8	4.1
tert-Butanol	0.5	7.3	16.6
	4.9	2.2	4.9
Isobutanol	0.5	3.6	8.0
	5.2	3.2	7.4

Tab. 6 *(continued)*

Analyte	Spiked concentration [mg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
		Ketones	
Acetone	8.3	5.9	13.4
	83.2	3.9	8.7
2-Butanone	0.2	8.1	18.4
	2.0	2.4	5.3
2-Pentanone	0.2	4.0	9.1
	2.0	3.7	8.3
3-Pentanone	0.2	3.3	7.6
	2.5	4.4	10.0
3-Methyl-2-butanone	0.3	5.9	13.2
	2.6	2.7	6.0
Cyclopentanone	0.2	8.0	18.0
	2.5	6.7	15.1
2-Hexanone	0.2	3.8	8.5
	2.0	4.5	10.1
3-Hexanone	0.2	5.7	13.0
	2.1	2.7	6.1
3,3-Dimethyl-2-butanone	0.2	5.5	12.4
	1.9	4.7	10.7
4-Methyl-2-pentanone	0.1	3.0	6.7
	1.2	1.8	4.1
Cyclohexanone	0.3	10.0	22.7
	2.6	4.2	9.4
2-Heptanone	0.2	7.2	16.3
	2.0	2.2	5.0
3-Heptanone	0.2	6.5	14.7
	2.0	3.7	8.5
4-Heptanone	0.2	7.6	17.1
	2.0	3.0	6.7
		Ethers	
MTBE	0.1	6.9	15.6
	1.0	2.4	5.3
THF	0.2	7.4	16.7
	2.2	2.0	4.5
1,4-Dioxane	0.2	7.0	15.9
	1.7	4.6	10.4

To determine day-to-day precision, urine samples were spiked with the analytes, processed and analysed. Eightfold determination yielded the precision data presented in Table 7.

Tab. 7 Day-to-day precision for the determination of the analytes in urine (n = 8)

Analyte	Spiked concentration [mg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Alcohols			
Methanol	2.9	2.9	7.0
	29.4	3.1	7.3
Ethanol	3.0	2.7	6.5
	30.2	2.6	6.2
1-Propanol	0.5	9.1	21.4
	5.3	4.1	9.8
2-Propanol	0.5	4.8	11.3
	5.4	1.4	3.2
1-Butanol	1.0	10.2	24.2
	9.9	4.4	10.5
2-Butanol	0.5	5.4	12.8
	5.3	3.8	9.0
tert-Butanol	0.5	3.2	7.5
	4.9	4.0	9.4
Isobutanol	0.5	4.8	11.3
	5.2	2.6	6.2
Ketones			
Acetone	8.3	7.0	16.5
	83.2	4.3	10.1
2-Butanone	0.2	3.6	8.5
	2.0	2.5	5.8
2-Pentanone	0.2	4.7	11.0
	2.0	2.3	5.4
3-Pentanone	0.2	1.9	4.5
	2.5	3.2	7.5
3-Methyl-2-butanone	0.3	6.4	15.2
	2.6	2.6	6.2
Cyclopentanone	0.2	3.9	9.1
	2.5	8.5	20.2
2-Hexanone	0.2	4.2	9.8
	2.0	4.9	11.5
3-Hexanone	0.2	4.9	11.5
	2.1	4.9	11.7
3,3-Dimethyl-2-butanone	0.2	1.9	4.5
	1.9	5.1	12.1
4-Methyl-2-pentanone	0.1	5.9	14.0
	1.2	8.1	19.1
Cyclohexanone	0.3	5.1	12.1
	2.6	4.4	10.5
2-Heptanone	0.2	3.4	8.1
	2.0	9.5	22.4
3-Heptanone	0.2	9.5	22.4
	2.0	10.6	25.2
4-Heptanone	0.2	8.6	20.3
	2.0	8.8	20.9

Tab. 7 *(continued)*

Analyte	Spiked concentration [mg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
		Ethers	
MTBE	0.1	9.2	21.8
	1.0	5.3	12.6
THF	0.2	4.8	11.4
	2.2	2.8	6.7
1,4-Dioxane	0.2	6.6	15.7
	1.7	4.6	10.8

11.2 Accuracy

Recovery experiments were performed to determine the accuracy of the method. To this end, urine samples were spiked with two different concentration levels of the analytes and analysed. The relative recovery rates were determined taking into account the background levels in urine. The results are summarised in Table 8.

Tab. 8 Relative recovery rates for the determination of the analytes in urine (n = 10)

Analyte	Spiked concentration [mg/l]	Recovery (rel.) r (mean value (range)) [%]	Standard deviation (rel.) s_w [%]
		Alcohols	
Methanol	2.9	94.3 (82.6–105)	9.1
	29.4	98.5 (93.3–101)	2.4
Ethanol	3.0	91.0 (76.9–103)	9.6
	30.2	101 (96.0–104)	2.5
1-Propanol	0.5	91.7 (75.9–101)	9.7
	5.3	97.6 (93.4–101)	2.4
2-Propanol	0.5	98.3 (94.4–104)	3.0
	5.4	98.7 (96.1–101)	1.8
1-Butanol	1.0	77.0 (70.5–88.6)	8.1
	9.9	98.6 (96.2–103)	2.2
2-Butanol	0.5	91.9 (81.0–97.9)	6.2
	5.3	94.1 (91.7–96.6)	1.8
tert-Butanol	0.5	93.9 (83.7–104)	7.3
	4.9	93.9 (90.8–96.3)	2.2
Isobutanol	0.5	102 (96.3–106)	3.6
	5.2	96.0 (93.1–102)	3.2

Tab. 8 *(continued)*

Analyte	Spiked concentration [mg/l]	Recovery (rel.) r (mean value (range)) [%]	Standard deviation (rel.) s_w [%]
	Ketones		
Acetone	8.3 83.2	116 (90.9–136) 107 (101–116)	11.4 4.2
2-Butanone	0.2 2.0	105 (85.7–115) 107 (101–110)	9.3 2.4
2-Pentanone	0.2 2.0	103 (87.4–108) 110 (105–116)	5.9 3.8
3-Pentanone	0.2 2.5	95.5 (92.3–103) 111 (102–117)	3.3 4.4
3-Methyl-2-butanone	0.3 2.6	98.8 (90.2–106) 110 (104–115)	5.9 2.7
Cyclopentanone	0.2 2.5	95.9 (87.0–112) 109 (99.1–118)	8.0 6.7
2-Hexanone	0.2 2.0	84.9 (87.7–89.3) 111 (104–122)	3.8 4.5
3-Hexanone	0.2 2.1	99.3 (89.6–109) 110 (105–114)	5.7 2.7
3,3-Dimethyl-2-butanone	0.2 1.9	92.1 (87.2–101) 109 (101–116)	5.5 4.7
4-Methyl-2-pentanone	0.1 1.2	112 (107–119) 92.8 (91.4–96.5)	3.0 1.8
Cyclohexanone	0.3 2.6	103 (92.0–123) 99.7 (91.6–108)	10.0 4.2
2-Heptanone	0.2 2.0	87.3 (80.7–102) 109 (101–118)	7.2 4.8
3-Heptanone	0.2 2.0	89.3 (80.5–103) 112 (103–117)	6.5 3.7
4-Heptanone	0.2 2.0	98.1 (86.6–114) 106 (102–113)	7.9 3.0
	Ethers		
MTBE	0.1 1.0	96.8 (84.2–105) 121 (114–124)	6.9 2.4
THF	0.2 2.2	90.6 (79.8–102) 113 (108–115)	7.4 2.0
1,4-Dioxane	0.2 1.7	86.4 (75.3–97.7) 98.6 (92.8–105)	7.0 4.6

11.3 Limits of detection and limits of quantitation

The limits of detection were estimated from the 3-fold signal-to-noise ratio and the limits of quantitation were determined accordingly (9-fold signal-to-noise ratio). The limits of detection and quantitation determined in this way are presented in Table 9.

Tab. 9 Limits of detection and limits of quantitation of the analytes

Analyte	Detection limit [mg/l]	Quantitation limit [mg/l]
Alcohols		
Methanol	0.2	0.6
Ethanol	0.1	0.3
1-Propanol	0.03	0.09
2-Propanol	0.02	0.06
1-Butanol	0.1	0.3
2-Butanol	0.05	0.15
tert-Butanol	0.05	0.15
Isobutanol	0.05	0.15
Ketones		
Acetone	0.01	0.03
2-Butanone	0.01	0.03
2-Pentanone	0.02	0.06
3-Pentanone	0.02	0.06
3-Methyl-2-butanone	0.01	0.03
Cyclopentanone	0.05	0.15
2-Hexanone	0.01	0.03
3-Hexanone	0.01	0.03
3,3-Dimethyl-2-butanone	0.01	0.03
4-Methyl-2-pentanone	0.01	0.03
Cyclohexanone	0.05	0.15
2-Heptanone	0.01	0.03
3-Heptanone	0.01	0.03
4-Heptanone	0.01	0.03
Ethers		
MTBE	0.005	0.015
THF	0.01	0.03
1,4-Dioxane	0.1	0.3

11.4 Sources of error

The described method permits the reliable determination of eight alcohols, fourteen ketones and three ethers in urine. The selection of the correct regression model for calibration poses a particular challenge. In the laboratory of the method developers, the use of the quadratic regression model provided reliable, precise results for all alcohols, the ether MTBE and for the ketone cyclohexanone over the entire concentration range. For the other ketones as well as for the two ethers 1,4-dioxane and THF, however, this regression model only yields valid results in the upper concentration range. Consequently, linear regression is used for these analytes in the lower concentration range (see Table 5). The need to use different calibration models may also be instrument-specific, in particular if high matrix samples are frequently analysed. Therefore, it must be checked in each individual laboratory and depending on the instrument, which calibration method gives valid results. For this method, the reliability of the

procedure has already been proven with day-to-day precision data, even over a longer period of time during which the instrument condition (due to matrix interference, etc.) may change.

It must be checked on a case-by-case basis whether it is deemed necessary to include internal standards. The external verification proved that even without ISTD addition very good validation data are achieved.

It is essential to include a reagent blank within each analytical run, since contamination can occur, for example, due to traces of analytes in the laboratory air. Any analyte concentrations in the reagent blanks have to be subtracted. After the injection of samples with high analyte concentrations (e.g. high calibration standards), carry-over effects may be observed in some cases. It is therefore advisable to inject water blanks at regular intervals and in any case after the injection of high calibration standards.

Acetone and 2-propanol elute shortly after each other with this method. At high analyte concentrations, the two peaks may therefore overlap partially. The resolution of the two peaks can be improved by adjusting the temperature gradient in the front range. However, at the same time, the analysis time increases and the peak shape of the subsequent analytes is slightly deteriorated. In the course of method verification, it could be shown that the use of an alternative analytical column (Zebron ZeZB-624 plus, 60 m × 0.32 mm × 1.8 µm by Phenomenex Ltd. Deutschland, Aschaffenburg, Germany, No. 7KM-G040-31) significantly improves the separation of acetone and 2-propanol, while the separation of the other analytes is similarly good.

12 Discussion of the method

The analytical method presented herein is suitable for the simultaneous determination of a total of 25 analytes in urine, including eight alcohols, fourteen ketones and three ethers. The method is both sensitive (limits of detection 0.005–0.2 mg/l) and reliable. The limits of detection of all analytes are at least ten times lower than the respective assessment values in urine (cf. Table 1) indicating that the present method enables a reliable monitoring of the given assessment values. It thus is a suitable method for the reliable determination of both occupational and environmental exposure to various alcohols, ketones and ethers. Characteristic is the calibration of all analytes using quadratic regression over the entire concentration range as well as calibration using linear regression for selected analytes in the lower concentration range (see Table 5). Using these two regression models, the analytical method provides very reliable results, especially in view of the large number of 25 analytes. Depending on the instrument used, it must be checked whether and for which analytes linear calibration or calibration using quadratic regression is more suitable. If necessary, further internal standards can easily be included in the method. For many analytes, isotope-labelled standards are commercially available. For methanol, ethanol, 2-propanol, acetone, 2-butanone, 4-heptanone and 2-pentanone, native background levels were observed in urine.

Instruments used Gas chromatograph with headspace autosampler and mass spectrometer by Agilent Technologies Deutschland GmbH, Waldbronn, Germany

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