The MAK Collection for Occupational Health and Safety

5-Fluorouracil – Determination of α-fluoro-β-alanine in urine by GC-MS/MS

Biomonitoring Method – Translation of the German version from 2019

R. Schierl[1], E. Fischer[1], G. Scherer[2], H.W. Hagedorn[2], T. Göen[3,*], A. Hartwig[4,*], MAK Commission[5,*]

1 *Method development, Institute and Outpatient Clinic for Occupational, Social, and Environmental Medicine, University hospital in Munich, Ziemssenstraße 1, 80336 München, Germany*

2 *External verification, ABF Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstraße 5, 82152 Planegg, Germany*

3 *Head of 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, Deutsche Forschungsgemeinschaft, Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute and Outpatient Clinic of Occupational, Social, and Environmental Medicine, Henkestraße 9–11, 91054 Erlangen, Germany*

4 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

5 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

***** *email: T. Göen (thomas.goeen@fau.de), A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords: 5-fluorouracil; α-fluoro-β-alanine; biomonitoring; urine; GC-MS/MS

Citation Note: Schierl R, Fischer E, Scherer G, Hagedorn HW, Göen T, Hartwig A, MAK Commission. 5-Fluorouracil – Determination of α-fluoro-β-alanine in urine by GC-MS/MS. Biomonitoring Method – Translation of the German version from 2019. MAK Collect Occup Health Saf [Original edition. Weinheim: Wiley-VCH; 2019 Jan;4(1):303–316]. Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2025. https://doi.org/10.34865/bi5121e2219_w

Republished (online): 30 Apr 2025

Originally published by Wiley-VCH Verlag GmbH & Co. KGaA; https://doi.org/10.1002/3527600418.bi5121e2219

Manuscript completed: 21 May 2015

Published (online): 30 Jan 2019

The commission established rules and measures to avoid conflicts of interest.

5-Fluorouracil – Determination of α-fluoro-β-alanine in urine by GC-MS/MS

Biomonitoring Methods

R. Schierl[1], E. Fischer[1], G. Scherer[2], H.-W. Hagedorn[2], Th. Göen[3,*], A. Hartwig[4,*], MAK Commission[5,*]

DOI: 10.1002/3527600418.bi5121e2219

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 verified the presented biomonitoring method.
The method described hereinafter permits the determination of α-fluoro-β-alanine (FBAL), the main metabolite of 5-fluorouracil, at low concentration levels and can be used for the biomonitoring of exposed individuals (manufacture, pharmacy, medicine).
To this end, urine samples are spiked with ^{13}C-FBAL as internal standard (IS) and acidified with 0.1 M hydrochloric acid. The samples are then extracted and purified using a strong cation exchanger. After resuspension and derivatisation, FBAL is analysed using GC-MS/MS in the MRM (Multiple Reaction Monitoring) mode and quantified with reference to the internal standard. Calibration standards are prepared in pooled urine and processed in the same way as the samples to be analysed.

Keywords

5-fluorouracil; α-fluoro-β-alanine; urine; biomonitoring; Analyses in Biological Materials; gas chromatography mass spectrometry; GC-MS/MS

Author Information

[1] Method development, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University hospital in Munich, Ziemssenstr. 1, 80336 München, Germany
[2] External verification, ABF – Analytisch-biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany
[3] Chair of the working group "Analyses in Biological Materials", Deutsche Forschungsgemeinschaft, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, Friedrich-Alexander University Erlangen-Nürnberg, Henkestraße 9–11, 91054 Erlangen, Germany
[4] Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Department of Food Chemistry and Toxicology, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany
[5] Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany

[*] Email: Th. Göen (thomas.goeen@fau.de), A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)

5-Fluorouracil – Determination of α-fluoro-β-alanine in urine by GC-MS/MS

Matrix:	Urine
Hazardous substance:	5-Fluorouracil
Analytical principle:	Capillary gas chromatography/tandem mass spectrometric detection (GC-MS/MS)
Completed in:	May 2015

Overview of the parameter that can be determined with this method and the corresponding hazardous substance:

Hazardous substance	CAS	Parameter	CAS
5-Fluorouracil	51-21-8	α-Fluoro-β-alanine (FBAL)	3821-81-6

Summary

The method described hereinafter permits the determination of α-fluoro-β-alanine (FBAL), the main metabolite of 5-fluorouracil, at low concentration levels and can be used for the biomonitoring of exposed individuals (manufacture, pharmacy, medicine).

To this end, urine samples are spiked with ^{13}C-FBAL as internal standard (IS) and acidified with 0.1 M hydrochloric acid. The samples are then extracted and purified using a strong cation exchanger. After resuspension and derivatisation, FBAL is analysed using GC-MS/MS in the MRM (Multiple Reaction Monitoring) mode and quantified with reference to the internal standard. Calibration standards are prepared in pooled urine and processed in the same way as the samples to be analysed.

Reliability data of the method

α-Fluoro-β-alanine (FBAL)

Within-day precision:	Standard deviation (rel.)	s_w = 1.7% or 0.9%
	Prognostic range	u = 3.8% or 1.9%
	at a spiked concentration of 5 or 40 µg FBAL per litre urine and where n = 10 determinations	

Day-to-day precision:	Standard deviation (rel.)	s_w = 4.6%
	Prognostic range	u = 10.3%
	at a spiked concentration of 40 µg FBAL per litre urine and where n = 10 determinations	
Accuracy:	Recovery rate (rel.)	r = 97.9% or 101%
	at a spiked concentration of 5 or 40 µg FBAL per litre urine and where n = 5 determinations	
Detection limit:	0.2 µg FBAL per litre urine	
Quantitation limit:	1.0 µg FBAL per litre urine	

General information on 5-fluorouracil

5-Fluorouracil is one of the most commonly used cytostatic drug to treat cancer. Exposure can therefore occur in many areas – ranging from production to preparation to administration to the patient. It belongs to the group of pyrimidine analogues and is mainly used to treat colorectal cancer and breast cancer. It is usually administered intravenously, but can also be given orally as a prodrug (Capecitabin®) in order to render its cytostatic effect as an active metabolite. Dermal application is also possible (Efudix®). More than 80% of the 5-fluorouracil administered is metabolised to FBAL in the liver in a number of steps and subsequently excreted in the urine (see Figure 1). The key enzyme seems to be the dihydropyrimidine dehydrogenase [Diasio and Harris 1989]. Hence, it is possible to specifically determine the internal exposure to this substance by determining the main metabolite FBAL. There is no known background exposure.

About 15% of the circulating cytostatic drug is filtered in the glomerulus and excreted in the urine, of which about 90% is excreted within the first hour. Only 1–3% of the originally circulating cytostatic drug is converted into the active metabolites 5-fluorodesoxyuridine monophosphate (FdUMP), 5-fluorouracil triphosphate (FUTP) and 5-fluorodesoxyuridine triphosphate (FdUTP), which are responsible for the cytostatic effect [Saif et al. 2009].

Since the elimination kinetics vary considerably from patient to patient, it is possible to control the effective concentration in the course of chemotherapy by determining FBAL in urine.

As 5-fluorouracil is administered in large scale, ambient monitoring of this substance has proved effective in detecting contamination in pharmacies, wards and outpatient clinics [Böhlandt and Schierl 2016]. If, however, unintentional incorporation of 5-fluorouracil is to be detected in staff (pharmacy, hospital), a sensitive analytical determination of FBAL in urine is necessary. Sessink et al. [1994] published a GC-MS method as early as 1994 and thus detected FBAL in the urine of a pharmaceutical plant worker (LOD 60 µg/L). In a study from France [Ndaw et al. 2010], urine samples from six pharmacy employees and 13 oncology nurses were analysed using LC-MS/MS (LOD 1 µg/L). 15–37% of 121 urine samples were found to be above the detection limit, with a maximum value of 22.7 µg/L. In 2006, Rubino et al. [2006] published a GC-MS method (LOD 20 µg/L) analysing 64 urine samples from hospital staff. In total, only three samples were above the detection limit, two contained approximately 30 µg FBAL/L, one even 1150 µg FBAL/L.

The new GC-MS/MS method presented herein with a detection limit of 0.2 µg/L was recently successfully applied in a study in which urine samples from oncology nursing staff were analysed for FBAL [Koller et al. 2018].

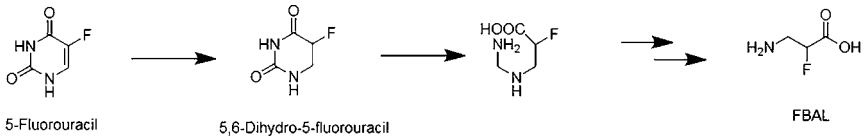

Figure 1: Postulated metabolism pathway of 5-fluorouracil to FBAL according to Bernadou et al. [1985].

Contents

1 General principles

The method described hereinafter permits the determination of α-fluoro-β-alanine (FBAL), the main metabolite of 5-fluorouracil, at low concentration levels and can be used for the biomonitoring of exposed individuals (manufacture, pharmacy, medicine).

To this end, urine samples are spiked with ^{13}C-FBAL as internal standard (IS) and acidified with 0.1 M hydrochloric acid. The samples are then extracted and purified using a strong cation exchanger. After resuspension and derivatisation, FBAL is analysed using GC-MS/MS in the MRM (Multiple Reaction Monitoring) mode and quantified with reference to the internal standard. Calibration standards are prepared in pooled urine and processed in the same way as the samples to be analysed.

2 Equipment, chemicals and solutions

2.1 Equipment

- Gas chromatograph with split/splitless injector, mass selective detector, backflush system, data processing system and autosampler
- Capillary gas chromatographic column: stationary phase: 5% phenyl methyl polysiloxane; length: 15 m; inner diameter: 0.25 mm; film thickness: 0.25 µm (e. g. VF-5 ms, Agilent, No. CP8939)
- Solid phase extraction cartridges: Chromabond® HR-XC 500 mg, 3 mL (e. g. Macherey-Nagel, No. 730955)
- Sealable containers for urine collection
- Heating block (e. g. Techne, No. DB-3A)
- Vortex mixer
- Analytical balance (e. g. Sartorius)
- Various volumetric flasks and measuring cylinders (e. g. Schott)
- 10 mL screw cap vials with plastic caps (e. g. Schuett)
- Nitrogen evaporator (e. g. Barkey)
- Variably adjustable microlitre pipettes (e. g. Eppendorf)
- Vials with inserts for gas chromatography (e. g. VWR)
- Urine monovettes (e. g. Sarstedt)
- SPE cartridge vacuum manifold (e. g. Macherey-Nagel)
- Centrifuge (e. g. Hettich)

2.2 Chemicals

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

- Acetonitrile (e. g. Merck, No. 1.14291)
- Ammonia solution, 25%, ultra pure (e. g. Roth, No. 5460)
- *N-tert*-butyldimethylsilyl-*N*-methyltrifluoracetamide (MTBSTFA, e. g. Aldrich, No. 394882)
- α-Fluoro-β-alanine (e. g. Campro, CS11-20093213)
- α-Fluoro-β-alanine-^{13}C (e. g. Campro, CS05-583_142)
- Hydrochloric acid 30% (e. g. Merck, No. 1.00318)
- Methanol (e. g. Merck, No. 1.06007)
- Helium 5.0 (e. g. Linde)
- Ultrapure water

2.3 Solutions

- 0.1 M hydrochloric acid
  About 80 mL ultrapure water are placed into a 100 mL volumetric flask and 1 mL of the 30% hydrochloric acid is added. The flask is then made up to the mark with ultrapure water.
- 15% hydrochloric acid
  Precisely 50 mL ultrapure water are placed into a 100 mL volumetric flask. The flask is then made up to the mark with 30% hydrochloric acid.
- 5% ammonia solution in methanol
  About 50 mL methanol are placed into a 100 mL volumetric flask and 20 mL of the 25% ammonia solution are added. The flask is then made up to the mark with methanol.

The diluted hydrochloric acid solutions are stable for at least six months when stored in a fume hood at room temperature. The ammonia solution is stable for at least two weeks when stored in a fume hood at room temperature.

2.4 Internal standard (IS)

- IS stock solution (400 mg/L)
  About 2 mg α-fluoro-β-alanine-^{13}C are weighed exactly into a 5 mL volumetric flask and dissolved in ultrapure water. The volumetric flask is then filled up with ultrapure water.

The stock solution is stored at –20 °C in a tightly sealed vial and is stable under these conditions for at least 24 months without significant losses.

- IS spiking solution (2 mg/L)
  50 µL of the IS stock solution are pipetted into a 10 mL volumetric flask. The flask is then filled up with ultrapure water.

The spiking solution is stored in the refrigerator at 4–6 °C and is stable for at least four weeks under these conditions.

2.5 Calibration standards

- Stock solution (400 mg/L)
  About 10 mg FBAL are weighed exactly into a 25 mL volumetric flask and dissolved in ultrapure water. The flask is then filled up to the mark with ultrapure water.

The stock solution is stored at –20 °C in a tightly sealed vial and is stable under these conditions for at least 24 months without significant losses.

- Spiking solution I (4 mg/L)
  About 5 mL ultrapure water are placed into a 10 mL volumetric flask and 100 µL of the stock solution are added. The flask is then filled up to the mark with ultrapure water.
- Spiking solution II (0.2 mg/L)
  About 5 mL ultrapure water are placed into a 10 mL volumetric flask and 500 µL of spiking solution I are added. The flask is then filled up to the mark with ultrapure water.

The spiking solutions are stored in the refrigerator at 4–6 °C and are stable for at least four weeks under these conditions.

The calibration standards are prepared in pooled urine. To prepare the pooled urine, urine samples from individuals not exposed to 5-fluorouracil are collected and mixed thoroughly. The pooled urine is stored at –20 °C and centrifuged at 1370 × *g* for 5 min prior to use. The calibration standards are prepared in 10 mL urine monovettes according to the pipetting scheme shown in Table 1.

The calibration standards are freshly prepared every working day.

Table 1: Pipetting scheme for the preparation of the calibration standards in pooled urine.

Calibration standard	Spiking solution	Volume of the spiking solution	Volume of the pooled urine	FBAL concentration
		[µL]	[µL]	[µg/L]
1	–	0	1000	0
2	II	10	990	2
3	II	25	975	5
4	II	50	950	10
5	II	100	900	20
6	I	25	975	100
7	I	50	950	200

3 Specimen collection and sample preparation

3.1 Specimen collection

The urine samples are collected in sealable containers (e. g. polypropylene), thoroughly mixed and aliquoted. 1 mL aliquots of the samples are stored at –20 °C. Under these conditions, the samples are stable for at least six months.

3.2 Sample preparation

For sample preparation, the 1 mL urine aliquots are thawed and thoroughly mixed and then each of them is acidified with 10 µL of the 15% hydrochloric acid. After adding 20 µL each of the IS spiking solution, the samples are extracted using strong cation exchanger cartridges (SPE).

To this end, the SPE cartridges are conditioned twice both with 2 mL methanol each and then with 2 mL ultrapure water each. It must be ensured that the cartridges do not run dry. Afterwards, the urine samples are transferred to the columns and are slowly drawn through the columns under vacuum. Each cartridge is then washed with 2 mL of 0.1 M hydrochloric acid, followed by 2 mL methanol, and well dried under vacuum. The analyte is eluted into 10 mL screw cap vials by a twofold elution with 1 mL of the 5% ammonia solution in methanol each. The samples are then evaporated to dryness under a stream of nitrogen. Afterwards, the residues are resuspended in 100 µL acetonitrile each. The samples are thoroughly mixed and 50 µL MTBSTFA are added to each sample for derivatisation. The sealed sample vials are incubated in a heating block at 70 °C for 20 min and then cooled to room temperature. The samples are mixed thoroughly and centrifuged at 1370 × *g* for 5 min. The supernatant is transferred to GC vials with microinserts and injected for analysis into the GC-MS/MS system.

4 Operational parameters

4.1 Gas chromatography

Capillary column:	Material:	Fused silica
	Stationary phase:	5% phenyl methyl polysiloxane (e. g. VF-5 ms)
	Length:	15 m
	Inner diameter:	0.25 mm
	Film thickness:	0.25 µm
Temperatures:	Column:	Initial temperature 70 °C, 1 min isothermal; increase at a rate of 25 °C/min to 150 °C, then increase at a rate of 8 °C/min to 190 °C, then backflush
	Injector:	250 °C
	Transfer line:	280 °C
Backflush:	Backflush for 1.1 min at –11.5 mL/min	
Carrier gas:	Helium 5.0	
Flow rate:	1.5 mL/min, constant	
Injection volume:	1 µL, pulsed splitless	

4.2 Mass spectrometry

Ionisation mode:	Electron ionisation (EI)
Ionisation energy:	70 eV
Detection mode:	MRM
Collision energy IS (Qual / Quant)	15 V/10 V
Collision energy FBAL (Qual / Quant)	15 V/5 V

All other parameters have to be optimised in accordance with the manufacturer's specifications.

5 Analytical determination

For analytical determination of FBAL in the urine samples prepared as described in Section 3, 1 µL each is injected into the GC-MS/MS system and analysed under the conditions specified. At least one quality control sample has to be included in each analytical run. The retention times of the analyte and of the internal standard as well as the corresponding ion traces are listed in Table 2.

The retention times given in Table 2 are intended to be a rough guide only. Users of the method must ensure proper separation performance of the analytical column used influencing the retention behaviour of the analytes. Figure 2 (in the Appendix) shows a chromatogram of a spiked and processed urine sample as an example.

If the measured value is above the calibration range (> 200 µg/L), the urine sample is to be diluted with pooled urine from non-exposed individuals and reanalysed.

Table 2: Retention times and detected ion traces.

Analyte	Retention time	Precursor ion	Product ion
	[min]	[*m/z*]	[*m/z*]
FBAL	8.367	278.1	100 116 144 (Quantifier)
IS	8.363	281.1	102 118 147 (Quantifier)

6 Calibration

The calibration standards prepared according to Section 2.5 are processed in the same way as the urine samples (Section 3) and analysed according to the operational conditions described in Sections 4 and 5. The calibration graph is obtained by plotting the ratio of the analyte peak area and the internal standard peak area against the spiked concentration. The slope of the calibration graph is calculated by linear regression. The calibration graph is linear up to a concentration of at least

200 µg/L (Figure 3 in the Appendix). In the case of consecutive analytical runs, it is usually sufficient to process one calibration standard as well as an unspiked pooled urine sample for calibration.

7 Calculation of the analytical results

The analyte concentration in a sample is determined by dividing the peak area of the analyte by the peak area of the internal standard. The ratio thus obtained is entered in the calibration function calculated according to Section 6. To this end, the axial intercept of the calibration graph is subtracted from the determined area ratio. The result is then divided by the slope of the calibration graph to give the analyte concentration in µg/L.

8 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, a quality control sample with a known and constant FBAL concentration is analysed within each analytical run. As material for quality control is not commercially available, it must be prepared in the laboratory.

To this end, pooled urine is spiked with a defined amount of FBAL, aliquoted and stored at –20 °C. The nominal value and the tolerance ranges of the quality control material are determined in a pre-analytical period (one analysis of the control material on 20 different days) [Bader et al. 2010]. The concentration level of the quality control samples analysed with each run should lie within the tolerance ranges obtained.

9 Evaluation of the method

The reliability of the method was confirmed by comprehensive validation and by implementation and validation of the method in an independent laboratory.

9.1 Precision

To determine within-day precision, pooled urine samples from non-exposed individuals were spiked with an FBAL standard solution, and then processed and analysed. The spiked FBAL concentrations were 5.05 µg/L and 40.4 µg/L. A ten-fold analysis of these urine samples yielded the within-day precision data shown in Table 3.

To determine day-to-day precision, pooled urine was spiked with 40.4 µg FBAL per litre urine. To this end, aliquots of the spiked urine sample were stored at –20 °C

Table 3: Within-day-precision for the determination of FBAL in urine (n = 10).

Analyte	Spiked concentration	Average concentration determined	Standard deviation (rel.) s_w	Prognostic range u
	[µg/L]	[µg/L]	[%]	[%]
FBAL	5.05	5.00	1.66	3.76
	40.4	39.3	0.85	1.92

Table 4: Day-to-day precision for the determination of FBAL in urine (n = 10).

Analyte	Spiked concentration	Average concentration determined	Standard deviation (rel.) s_w	Prognostic range u
	[µg/L]	[µg/L]	[%]	[%]
FBAL	40.4	40.3	4.55	10.3

Table 5: Relative recovery rates for the determination of FBAL in urine (n = 5).

Analyte	Spiked concentration	Mean rel. recovery r	Range
	[µg/L]	[%]	[%]
FBAL	5.05	97.9	88.3–104
	40.4	101	96.5–105

and processed and analysed on ten different days. The day-to-day precision data thus obtained are shown in Table 4.

9.2 Accuracy

Recovery experiments were performed to determine the accuracy of the method. To this end, pooled urine from non-exposed individuals was spiked with FBAL at concentrations of 5.05 µg/L and 40.4 µg/L, aliquoted and analysed. The recovery rates obtained are summarised in Table 5.

In addition, this method was compared with an LC-MS/MS method for the determination of FBAL in urine, which was developed and published by another working group [Ndaw et al. 2010]. That working group provided the author of this method with four native urine samples for the purpose of comparison. The results (Table 6) substantiate the comparability of the two methods.

Table 6: Comparison of the analytical results for the determination of FBAL in urine by LC-MS/MS and GC-MS/MS.

Sample	LC-MS/MS	GC-MS/MS
	[µg/L]	[µg/L]
1	7.8	5.5
2	28.1	28.3
3	59.8	40.8
4	131	178

9.3 Limit of detection and limit of quantitation

The detection limit and the quantitation limit were estimated on the basis of the 3-fold and the 10-fold signal-to-noise ratio, respectively. Under the given conditions of specimen preparation and analytical determination, the detection limit and quantitation limit for the determination of FBAL in urine were 0.2 µg/L and 1.0 µg/L, respectively.

9.4 Sources of error

As the isotopic purity of the internal standard is only 98%, the addition of the IS always results in a low FBAL background signal in the analysed samples. For this reason, it is absolutely necessary to include a reagent blank in each analytical run in order to take the background level of the analyte in the urine into account. Urine samples with high creatinine levels occasionally showed interfering signals in the chromatogram, which, however, could easily be separated from the analyte peak. In principle, the method can also be applied without a backflush system. Due to the higher matrix load, however, the detection limit may be slightly higher.

10 Discussion of the method

The analytical method presented herein permits the precise and sensitive determination of FBAL in urine by GC-MS/MS and is particularly suitable for the determination of the internal load after accidental exposure to 5-fluorouracil.

The external verification scientist successfully implemented the method with minor modifications and was able to confirm the validation data at large. However, the method was verified without the use of a backflush system. Due to the higher matrix load, this led to a slightly higher quantitation limit of 2 µg/L. In addition, the urine samples were diluted with 1 mL of water prior to solid phase extraction when verifying the method, which improved the sample flow through the SPE cartridges considerably. Alternatively, the samples can also be evaporated after solid phase extraction using a vacuum centrifuge.

Instruments used Agilent GC-MS/MS-system with split/splitless injector, mass selective detector, backflush system, data processing system and autosampler.

11 References

Bader M, Barr D, Göen Th, Schaller KH, Scherer G, Angerer J, Working group Analyses in Biological Materials (2010) Reliability criteria for analytical methods. In: Angerer J and Hartwig A (Eds): The MAK-Collection for Occupational Health and Safety Part IV: Biomonitoring Methods, Vol. 12, Wiley-VCH, Weinheim
https://doi.org/10.1002/3527600418.bireliabe0012

Bernadou J, Armand JP, Lopez A, Malet-Martino MC, Martino R (1985) Complete Urinary Excretion Profile of 5-Fluorouracil during a Six-Day Chemotherapeutic Schedule, as Resolved by ^{19}F Nuclear Magnetic Resonance. Clin Chem 31: 846–848

Böhlandt A and Schierl R (2016) Benefits of Wipe Sampling: Evaluation of Long-Term 5-Fluorouracil and Platinum Monitoring Data. Pharm Technol Hosp Pharm 1: 139–150

Bundesärztekammer (2014) Richtlinien der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen. Dt Ärztebl 111: A1583–1618

Diasio RB and Harris BE (1989) Clinical pharmacology of 5-fluorouracil. Clin Pharmacokinet 16: 215–237

Koller M, Böhlandt A, Haberl C, Nowak D, Schierl R (2018) Environmental and biological monitoring on an oncology ward during a complete working week. Toxicol Lett 298: 158–163

Ndaw S, Denis F, Marsan P, D'Almeida A, Robert A (2010) Biological monitoring of occupational exposure to 5-fluorouracil: Urinary alpha-fluoro-betaalanine assay by high performance liquid chromatography tandem mass spectrometry in health care personnel. J Chromatogr B 878: 2630–2634

Rubino FM, Verduci C, Buratti M, Fustinoni S, Campo L, Omodeo-Salè E, Giglio M, Iavicoli S, Brambilla G, Colombi A (2006) Assay of urinary alpha-fluoro-beta-alanine by gas chromatography-mass spectrometry for the biological monitoring of occupational exposure to 5-fluorouracil in oncology nurses and pharmacy technicians. Biomed Chromatogr 20: 257–266

Saif MW, Syrigos KN, Katirtzoglou NA (2009) S-1: a promising new oral fluoropyrimidine derivative. Expert Opin Investig Drugs 18: 335–348

Sessink PJ, Timmermanns JL, Anzion RB, Bos RP (1994) Assessment of occupational exposure of pharmaceutical plant workers to 5-fluorouracil. Determination of alpha-fluoro-beta-alanine in urine. J Occup Med 36: 79–83

Method development: R. Schierl, E. Fischer
External verification: G. Scherer, H.-W. Hagedorn
Chair of the working group "Analyses in Biological Materials", Deutsche Forschungsgemeinschaft: Th. Göen
Chair of the "Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area", Deutsche Forschungsgemeinschaft: A. Hartwig
Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft: MAK Commission

12 Appendix

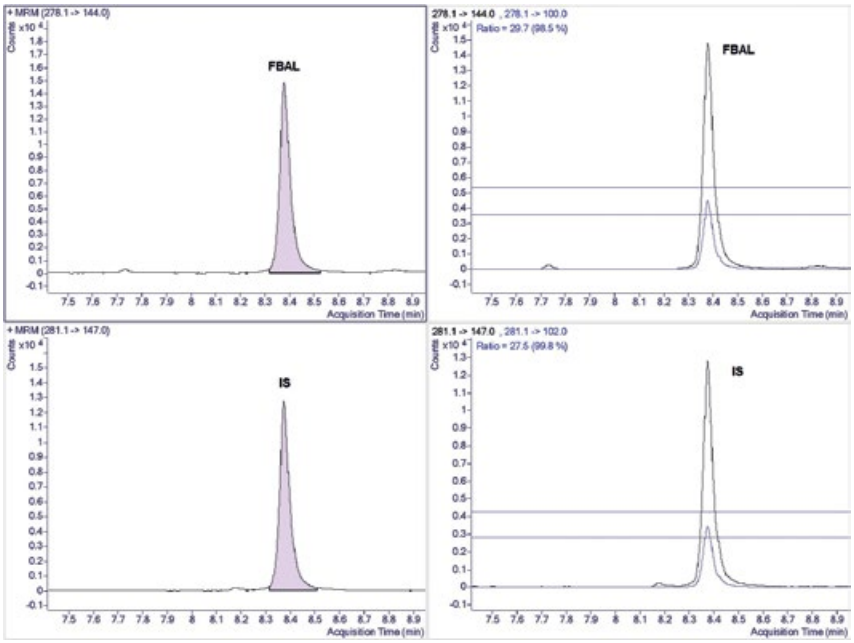

Figure 2: Chromatogram of a processed urine sample at a spiked concentration level of 40.4 µg FBAL per litre.

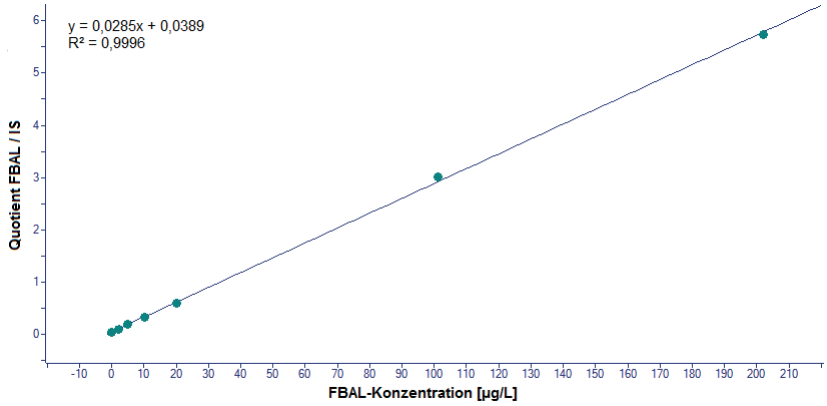

Figure 3: Example of a calibration curve for FBAL in urine.