# Simple, selective, and sensitive measurement of urea in body fluids of mammals by reversed-phase ultra-fast liquid chromatography

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**ABSTRACT**: Ultra-fast liquid chromatography with a photodiode array detector for simple and rapid determination of urea in body fluids of farm animals is described. Blood plasma, milk, and urine samples are treated with trichloroacetic acid and then centrifuged. Supernatants are derivatized at room temperature using *p*-dimethylaminobenzaldehyde. Samples are separated using a ternary gradient of methanol in buffer and water. Derivatized urea in standards and biological samples is analyzed using a Phenomenex $C_{18}$-column (Synergi 2.5 μm, Hydro-RP, 100Å, 100 × 2 mm). The photodiode detector is set to 370 and/or 254 nm for detection. Temperature is maintained at 27°C by a column heater. Clear separation of derivatized urea from the endogenous species present in assayed biological samples was achieved in less than 6 min. The urea adduct peak eluted at 4.36 ± 0.05 min. Average recoveries of the urea standards added to assayed biological materials were satisfactory (i.e. 100.2 ± 4.1%). Our chromatographic method with photodiode detection at 370 nm and at 254 nm, in particular, offers low detection ($L_D$) and quantification limits ($L_Q$) ($^{370nm}L_D$ = 0.47 ng, $^{254nm}L_D$ = 0.027 ng and $^{370nm}L_Q$ = 1.41 ng, $^{254nm}L_Q$ = 0.080 ng, respectively). Our liquid chromatography based on detection at 370 nm is the most versatile analytical tool that assures sensitive, accurate, and precise analysis of urea in urine, milk, and plasma samples, and selected diets for mammals. The presented chromatographic procedure is especially suitable for preparation of reference sample sets, very accurate and precise research purposes or rapid clinical diagnostic with smaller sample sets. Urea in urine can be also determined using our liquid chromatography with detection at 254 nm. Detection of urine urea at 254 nm is more sensitive and precise compared with monitoring at 370 nm. Our chromatographic method based on photodiode detection at 370 nm and especially at 254 nm is suitable for the non-invasive analysis of urea in only urine of humans and animals.

**Keywords**: urea; derivatization; liquid chromatography; milk; blood plasma; urine

Urea is the main and final catabolite of nitrogen compounds in humans and animals; its level often provides information on the nutritional status of monogastric and ruminal animals (Ferguson, 2000; Francis et al., 2002; Jílek et al., 2006; Sejrsen at al., 2006; Zhai et al., 2006; Řehák et al., 2009). On the other hand, creatinine, formed from phosphocreatine and creatine, is a marker of the amino acid pool and its generation reflects the slow turnover of muscle protein. These two final catabolites are used as biomarkers of many low- and intermediate-molecular mass toxic solutes accumulated in the blood of humans with diminished renal function (Gutiérrez et al., 2008). The majority of clinical laboratory tests used to assess kidney function are related to the measurement of the kidney ability to clear waste products, usually nitrogenous species like creatinine and urea, from living organisms. Urea is also a bioindicator of liver function; its quantification in physiological fluids is an impor-

tant and frequently requested task in clinical laboratories (Czauderna and Kowalczyk, 2009; Dhawan et al., 2009). Moreover, milk urea nitrogen can be a practical bioindicator of protein utilization by lactating cows in dairy cattle nutrition programs (Petersom et al., 2004). Unfortunately, sensitive and accurate determination of urea in urine, and especially in milk and dairy products, is hindered by the opacity of milk and variation in its fat content (Carlsson and Bergström, 1994). Quantitative determination of urea is important in a wide range of fields, such as environmental monitoring and food science. Indeed, accurate and precise determination of urea concentrations in cattle or sheep can help satisfactorily meet nutritional requirements, increase meat and milk production, as well as improve reproductive performance and decrease nitrogen pollution of the environment (Petersom et al., 2004). A variety of analytical methods is available for the routine analysis of urea, such as colorimetric direct methods, the specific Ureakvant method, enzymatic flow injection analysis, and indirect infrared methods (e.g. Anadis FT MI600, Bentley B2000, Foss MikoScan) (Yatzidis et al., 1964; Oltner et al., 1985; Sheppard et al., 1996; Francis et. al., 2002; Hanuš et al., 2008; Hering et al., 2008; Dhawan et al., 2009). The most popular method for determination of urea involves the use of urease to enzymatically catalyse the hydrolysis of urea, followed by detection of ammonia by colour reactions or electrochemical techniques, or the derivatization of urea with butane-2,3-dione monoxime, or other reagents, to form species containing a chromophore (Francis et al., 2002; Sehitoğullari and Uslan, 2002; Gutiérrez et al., 2008). The enzyme-related approach is, however, unsatisfactory for the accurate, precise and selective quantification of urea. Moreover, the mentioned methods are time consuming. On the other hand, ultra-fast liquid chromatography (UFLC) and high-performance thin layer chromatography-densitometry chemical methods have the advantage of being more specific and accurate (Dallet et al., 2000; Clark et al., 2007; Czauderna and Kowalczyk, 2009). Liquid chromatography methods are usually the most selective and precise analytical tools and utilize widely available and relatively inexpensive $C_{18}$ hydrophobic columns. As urea is extremely poorly retained on these columns, before being subjected to liquid chromatography with photodiode detection (DAD) it should be converted into a derivative containing a chromophore. Thus, the aim of this study was to improve efficient pre-column derivatization of urea, as well as gradient conditions for UFLC analysis of urea, particularly in blood plasma, milk, and urine of domestic animals. It seemed desirable to develop a new, simple ultra-fast liquid chromatography method for separation of derivatized urea using procedures that enable accurate, precise, and sensitive determination of urea in urine, blood plasma, milk, and selected diets of humans and farm animals.

## MATERIAL AND METHODS

### Chemicals

Super-gradient HPLC methanol was purchased from Lab-Scan (Eire, Ireland); urea, trichloroacetic acid (TCA), super gradient HPLC tetrahydrofuran (THF), and *p*-dimethylaminobenzaldehyde (DMAB) were obtained from Sigma (St. Louis, USA). All other chemicals used were of analytical reagent grade (POCH S.A.; Gliwice, Poland). Water used for the preparation of mobile phases and solutions of chemicals was purified using an ElixTM water purification system (Millipore, Toronto, Canada).

### Sample preparation and derivatization procedure

Based on the systematic optimization of the derivatization reaction conditions, the following highly efficient derivatization procedure for urea in biological materials and in urea standard solutions is briefly presented below. To a 100 µl portion of cooled ( ≈ 0°C) body fluids (blood plasma, urine, or milk), 50 µl of a cooled ( ≈ 0°C) 25% aqueous TCA solution (m/m) were added and the resulting mixture was centrifuged at ca. 5000 *g* for 10 min at 4°C. The obtained supernatant was used for the derivatization procedure. To 75 µl of the obtained supernatant, 25 µl of water were added. If the volume of the assayed supernatant is larger (up to 100 µl), the volume of the added water should be smaller, so that the final volume of the resulting solution is 100 µl. Then, to 100 µl of the resulting solution, 25 µl of DMAB (680 mg DMAB in 5 ml of 20% HCl) were added. The obtained solution was vigorously mixed and reacted for 6–7 min at room temperature. It is recommended to protect the processed solutions from light. The derivati-

zation procedure for urea standards (2.6–260 µg) was the same as for biological samples. A 0.5–50 µl sample of the resulting solution was injected onto a reversed phase column.

The analyses of urea in 100–150 mg of finely powdered samples of animal diets (e.g. soya bean meal, cereal bran) were performed after three or four extractions with 1 ml of 25% aqueous TCA solution (m/m) (Czauderna and Kowalczyk, 2009). The resulting extract was centrifuged at ca. 5000 g for 10 min at 4°C. Water from the obtained supernatant was removed at 40–60°C under a stream of nitrogen or argon. The obtained residue was re-dissolved in 50 µl of water and 25 µl of 25% aqueous TCA solution (m/m). The resulting solution was used for derivatization as above.

## Chromatographic equipment, conditions and gradient composition

An ultra-fast liquid chromatography (UFLC) system (SHIMADZU, Kyoto, Japan), incorporating two LC-20ADXR liquid chromatographic pumps ($UFLC_{XR}$), a SIL-$20AC_{XR}$ autosampler ($LFLC_{XR}$), a CBM-20A communications bus module (UFLC), a CTO-20A column oven, a DGU-$20A_5$ degasser, and a SPD diode array detector (DAD), was used in this study. A sensitive DAD, was equipped with a 10 µl flow cell. The DAD was operated in the UV range of 190–600 nm with a measurement frequency of 1 spectrum per s and spectral resolution of 1.2 nm. The column used was a Phenomenex $C_{18}$-column (Synergi 2.5 µm, Hydro-RP, 100 Å, 100 × 2 mm). A guard column, containing $C_{18}$ pellicular packing material (Phenomenex $C_{18}$; 5 × 4 mm), was placed in front of the analytical column for protection. A column heater maintained the temperature at 27°C. The autosampler thermostat was set to 25°C or 30°C.

Samples were analysed using a linear ternary gradient of methanol in a buffer and water (Table 1). The minimal and maximal system pressures were 41.5 ± 0.1 MPa and 58.9 ± 0.1 MPa, respectively. Solvent A consisted of methanol, solvent B consisted of 0.09 M phosphate buffer and THF (99:1, v/v), while solvent C was water. Phosphate buffer was prepared from $Na_2HPO_4$ adjusted to pH 7.0 with 50% phosphoric acid. Mobile phases were filtered through a 0.45 µm membrane filter (Millipore, Billerica, USA) and then degassed for 2–3 min prior to liquid chromatography.

The derivatized urea peak was identified on the absorption spectra by comparing the retention times of processed urea standards that were injected separately and by adding urea standard solutions to processed biological samples. The limit of detection ($L_D$) was calculated at a signal-to-background ratio of 3, and the limit of quantification ($L_Q$) was defined as 10 times the background under a peak (Lin and McKeon, 2005).

## RESULTS AND DISCUSSION

To avoid the problems caused by overlapping of the peaks of urea and endogenous compounds present in the assayed biological materials, urea was derivatized with DMAB prior to chromatographic separation in order to obtain an adduct possessing an aromatic chromophore of high molar absorption at longer UV wavelengths. As expected, DMAB converted urea to an adduct with two wide UV bands in the spectral ranges of 220–280 nm and

Table 1. Ternary gradient elution program[a] used for UFLC-DAD analysis of derivatized urea

| Time (min) | Flow rate (ml/min) | Composition (%) | | |
|---|---|---|---|---|
| | | solvent A (methanol) | solvent B (buffer) | solvent C (water) |
| 0 | 0.4 | 30 | 70 | 0 |
| 5.8 | 0.4 | 30 | 70 | 0 |
| 6.0 | 0.4 | 30 | 0 | 70 |
| 6.5 | 0.4 | 100 | 0 | 0 |
| 11.0[b] | 0.9 | 100 | 0 | 0 |

[a]all changes of solvent compositions were linear

[b]after 11 min the column was re-equilibrated for 7 min in 30% solvent A and 70% solvent B at a flow rate of 0.4 ml/min

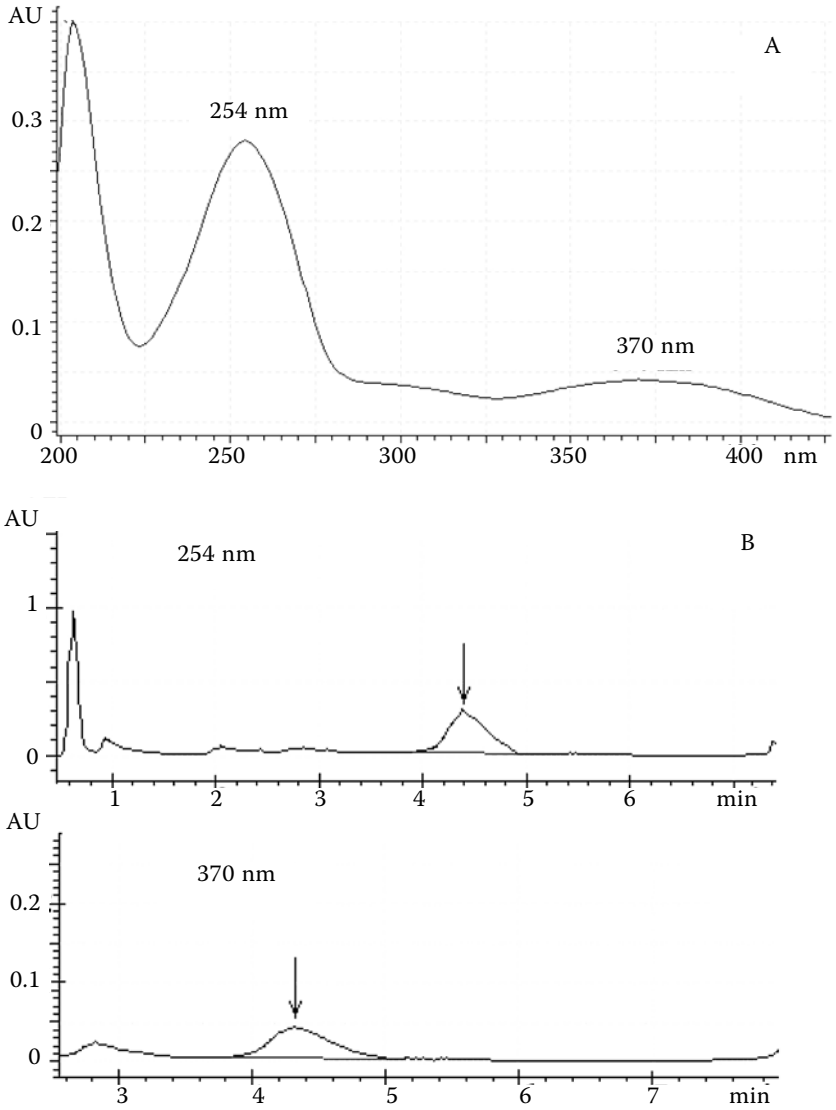


Figure 1. Liquid chromatography of derivatized urea in a standard. **A** – typical UV spectrum of a derivatized urea standard; **B** – chromatogram of 20 µg of a processed urea standard (upper chromatogram: UV detection at 254 nm; lower chromatogram: UV detection at 370 nm). Injection volume of a processed standard was 15 µl. Arrows indicate the elution time for derivatized urea (4.36 ± 0.05 min)

340–460 nm; these bands were due to the presence of the aromatic ring with lone electron pairs (Figure 1A). High molar absorptivity and the occurrence of absorption maxima close to 254 nm and 370 nm, in particular, made this derivative almost ideally suited for analysis with UV detection. Moreover, the derivatized urea was substantially retained on a long column packed with a strongly hydrophobic bonded phase ($C_{18}$) (Figure 1B).

To achieve the highest possible derivatization yield, the derivatization of urea in standards and biological samples was performed using the smallest possible volumes of reagent solvents and large excess of DMAB. Therefore, in order to minimize precipitate formation after this highly efficient derivatization, processed urea standards, ovine or goat blood plasma, and ovine or goat urine samples should be stored at 25°C in an autosampler before injection onto the HPLC column, whereas processed ovine and goat milk samples should be stored at 30°C in an autosampler before injection onto the column. Our detailed study documented that optimum separation of the derivatized urea peak from endogenous species present in all the assayed biological materials was obtained using phosphate buffer adjusted to pH 7, the proposed gradient elution program, and photodiode detection. As expected, the urea peak was absent from the blank when the proposed gradient elution program and photodiode detection at 254 and/or 370 nm were applied. Moreover, no urea peak was observed when the blood plasma, milk, and urea samples were processed in the absence of the derivatizing reagent followed by UFLC analyses

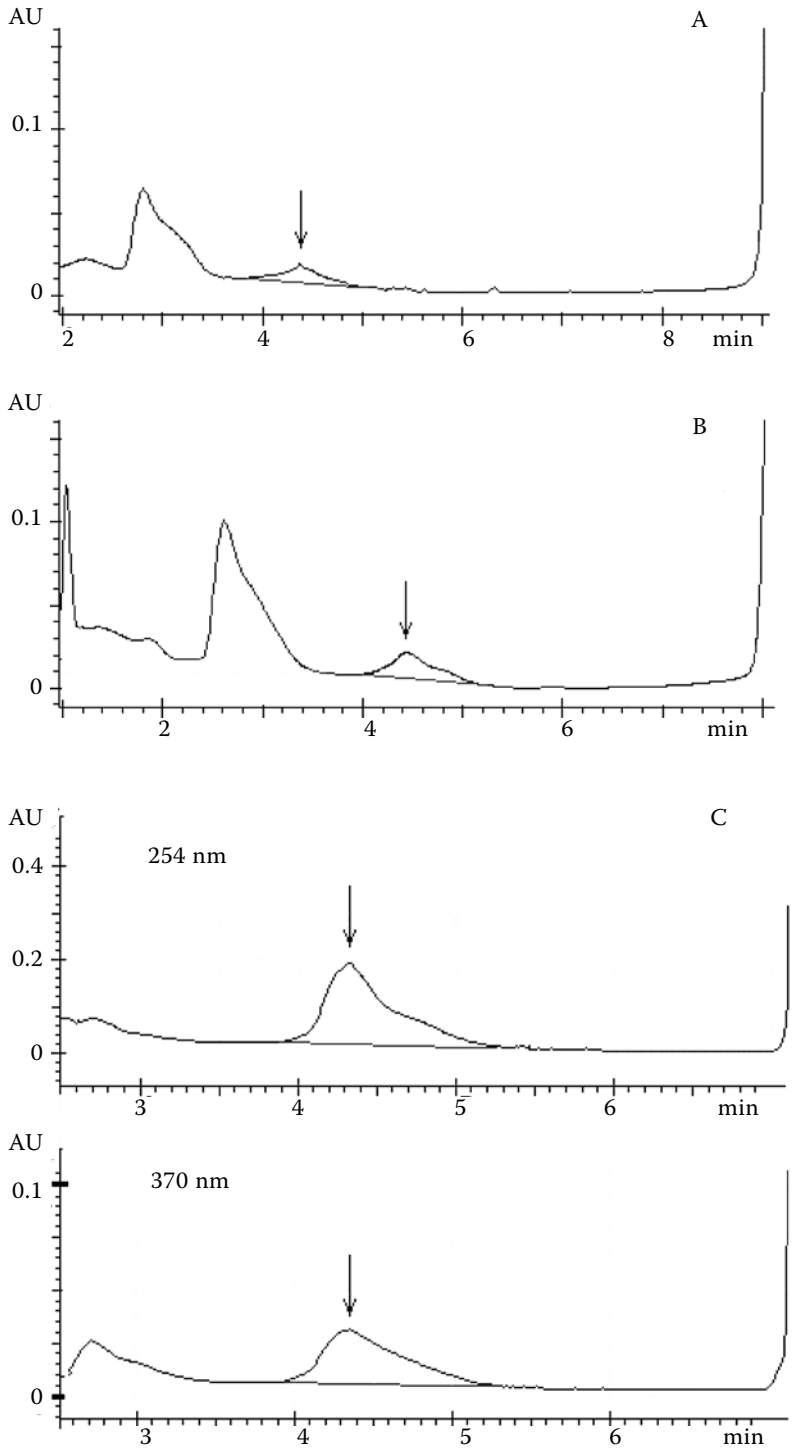


Figure 2. Liquid chromatography of derivatized urea in biological materials. **A** – ovine blood plasma chromatogram (UV detection at 370 nm); **B** – ovine milk chromatogram (UV detection at 370 nm); **C** – ovine urine chromatogram (upper chromatogram: UV detection at 254 nm; lower chromatogram: UV detection at 370 nm). Injection volumes of processed samples were 15–20 µl. Arrows indicate the elution time for derivatized urea (4.36 ± 0.05 min)

with photodiode detection at 254 and/or 370 nm. On the other hand, liquid chromatography with photodiode detection at 254 nm resulted in poor separation of the derivatized urea peak as numerous endogenous substances present in the assayed milk and blood plasma samples possessed significant absorptivity in the UV range of 200–340 nm. Clear separation of urea from endogenous species present in the blood plasma and milk samples was achieved using photodiode detection at 370 nm (Figures 2A and 2B). On the other hand, in urine samples, endogenous species do not interfere with the derivatized urea peak as these species have negligible absorbance above 235 nm. Therefore,

our derivatization procedure and liquid chromatography permitted quantification of urea in urine samples using photodiode detection at 254 nm as well as at a longer wavelength (i.e. at 370 nm) (Figure 2C). As can be seen in Figure 2, the satisfactory selectivity and suitable quantification of derivatized urea in the assayed biological samples were achieved by using the proposed gradient elution program and photodiode detection at 370 nm. Thus, our liquid chromatography based on photodiode detection at 370 nm, is the most versatile analytical tool that assures satisfactory analysis of urea in all assayed biological materials. Only urea in urine samples can be also satisfactorily analysed using proposed liquid chromatography with photodiode detection at 254 nm (Figure 2C). Figures 1 and 2 documented that in our liquid chromatography, the urea adduct peak eluted at 4.36 ± 0.05 min.

The stability of derivatized urea in the standards and all the processed biological samples was investigated with respect to the storage time of the processed standards and assayed biological materials. Our studies documented that the areas of the derivatized urea peaks were practically equal if the processed urea samples were protected from light and stored at 10°C for 8 h, while the processed blood and milk samples were stored at 18°C for 7 h. Obviously, before injection onto the column, the processed urine and plasma samples should be warmed to 25°C, while the processed milk samples to 30°C.

## Evaluation of the new derivatization method

The analytical problem in the current study was to achieve as rapid as possible separation of derivatized urea from all endogenous species present in the assayed biological materials and to decrease the background under the derivatized urea peak as well as the noise level in its proximity.

An important analytical problem addressed in the current study was to develop a suitable separation technique for the derivatized urea to avoid the interference of endogenous substances present in all assayed biological materials. Therefore, different monitoring wavelengths were applied to ensure that the species present in the assayed samples did not interfere with the separation efficiency of the derivatized urea peak. The purity ($P_{\%}$) of the derivatized urea peak in the analysed biological materials was assessed by determining the relationships between the monitoring wavelength ($\lambda_{nm}$) and the ratios (R) of the derivatized urea peak areas ($S_{sample}$) in the biological samples ($R_{sample}$) and comparing them to the derivatized urea peak area ($S_{standard}$) in the processed urea standards ($R_{standard}$), i.e., $P_{\%} = (R_{sample}/R_{standard}) \times 100\%$ (Czauderna and Kowalczyk, 2001). The values of $R_{standard}$ and $R_{sample}$ were calculated using the relationship between the derivatized urea peak area monitored at 254 nm ($S_{max\ standard}$ and $S_{max\ sample}$, respectively), and the derivatized urea peak areas in the standard ($S_{standard}$) and biological samples ($S_{sample}$) obtained at wavelengths ranging from 230–425 nm (i.e., $R_{standard} = S_{standard}/S_{max\ standard}$; $R_{sample} = S_{sample}/S_{max\ sample}$). Purity analyses showed that the derivatized urea peak for ovine blood plasma and ovine milk samples was satisfactorily pure (i.e. 98.2 ± 2.9%) and free from the influence of closely located signals of unidentified species at wavelengths ranging from 350 to 400 nm. We suggest that difficulties in the accurate integration of the small peak of derivatized urea in plasma and milk samples at longer UV ranges ($\lambda > 400$ nm) were responsible for the poor purity of the derivatized urea peak in processed plasma and milk samples. On the other hand, the derivatized urea peak in ovine and goat urine samples is satisfactorily pure (nearly 100%) at wavelengths ranging from 238 to 425 nm, as the derivatized urea peak in processed urine samples is usually approx. 10 times larger compared with this peak in plasma and milk samples.

The reliability of the current method was assessed by the addition of an urea standard solution to the assayed ovine urine samples. The recoveries of added urea standards (i.e. 12, 25, and 36 μg) to the processed ovine urine samples were satisfactory for photodiode detection at 254 nm, i.e. 101.7 ± 3.8% ($n$ = 5), 102.6 ± 4.1% ($n$ = 3) and 101.2 ± 3.4% ($n$ = 3), respectively. Similar values of recoveries of urea added to the urine samples were found for detection at 370 nm. However, photodiode detection at 254 nm is preferred to urea quantification in urine samples, as the detector response at 254 nm is ca. 6 times greater compared with detection at 370 nm (Table 2).

The recovery of 4 μg of an urea standard added to an ovine plasma sample was satisfactory for UV monitoring at 370 nm (i.e. 98.7 ± 3.2%, $n$ = 3), while the recovery of 9.6 μg of an urea standard added to a goat milk sample was 98.4%. Thus, our ultra-fast liquid chromatography with photodiode detection at 370 nm gave good accuracy of urea assays in

Table 2. Relative standard deviation (RSD), linear regression curve and correlation coefficients (*r*) derived from quantification of derivatized urea in urea standard, urine, milk, and blood plasma samples

| Processed sample | Monitoring wavelength (nm) | RSD (%) | Linear regression curve | Correlation coefficient *r* |
|---|---|---|---|---|
| Urea standard | 254 | 1.42 | $y$ (μg) = $6.097 \times 10^{-6}$ $S_n = 0.91$[a] | 0.9994 |
| | 370 | 1.54 | $y$ (μg) = $4.080 \times 10^{-5}$ $S_n = 0.59$[a] | 0.9989 |
| Ovine plasma | 370 | 2.31 | $y$ ($S_n$) = $1.622 \times 10^{-4}$ $V_{\mu l} = 0.37$[b] | 0.9961 |
| Goat milk | 370 | 2.44 | $y$ ($S_n$) = $1.465 \times 10^{-4}$ $V_{\mu l} = 0.21$[b] | 0.9937 |
| Ovine urine[c] | 254 | 1.74 | $y$ ($S_n$) = $1.032 \times 10^{-6}$ $V_{\mu l} = 0.11$[b] | 0.9984 |
| | 370 | 1.96 | $y$ ($S_n$) = $6.614 \times 10^{-6}$ $V_{\mu l} = 0.09$[b] | 0.9968 |

[a]$y$ (μg) and $S_n$ are the amount of urea (μg) in a processed standard and the derivatized urea peak area, respectively; the urea concentration in processed standards was 2.6–260 μg

[b]$y$ ($S_n$) and $V_{\mu l}$ are the derivatized urea peak area and volumes (μl) of analyzed biological samples

[c]average ratio of the urea peak areas monitored at 254 nm ($^{254nm}S_n$) and 370 nm ($^{370nm}S_n$) is 6.42

processed plasma and milk samples. Furthermore, our detailed urea peak purity analyses showed that our derivatization method followed by liquid chromatography with detection at 370 nm enabled satisfactory quantification of urea in feed samples (e.g. cereal bran and soya bean meal).

The reproducibility of the present method was assessed by performing replicate injections of 5.2 μg of processed urea standards ($n$ = 6), ovine urine ($n$ = 6), goat milk ($n$ = 4), and ovine plasma samples ($n$ = 7); the obtained values of relative standard deviation (RSD) are presented in Table 2. Moreover, reproducibility of the current method was also assessed by analyzing the linearity of urea quantification in urea standard solutions as well as in urine, milk, and plasma samples. As can be seen from results summarized in Table 2, the proposed method offers satisfactory precision as RSD (%) values are low for processed urea standards and assayed biological samples. Moreover, experiments showed that liquid chromatography with detection at 370 nm offered satisfactory linearity of derivatized urea quantification in urea standard solutions and in all assayed biological samples, as the correlation coefficients ($r$) are close to 1 (Table 2). Detection at long wavelengths, i.e. 254 and 370 nm, offers very low detection limits ($L_D$) and quantification limits ($L_Q$) ($^{254nm}L_D$ = 0.027 ng, $^{370nm}L_D$ = 0.47 ng and $^{254nm}L_Q$ = 0.080 ng, $^{370nm}L_Q$ = 1.41 ng, respectively).

Despite a weaker response of the photodiode detector at 370 nm than at 254 nm (Table 2, Figures 1B and 2 C), $L_D$, $L_Q$ and RSD clearly show that the proposed ultra-fast liquid chromatography with UV monitoring at 370 nm offers excellent sensitivity and precision and allows identification and quantification of urea in blood plasma, milk and urine samples if one takes into account original contents of urea in plasma (range: 6–19 mg/100 ml) (Ferguson, 2000; Sejrsen et al., 2006), milk (range: 2.3–23 mg/100 ml) (Ferguson, 2000) and urine (range: 1–2 g/100 ml) (Sejrsen et al., 2006) samples. Considering the above, we argued that our ultra-fast liquid chromatography with photodiode detection (UFLC-DAD) allows very sensitive, accurate and precise quantification of urea in processed biological materials. Indeed, the highest possible concentrations of trichloroacetic acid, *p*-dimethylaminobenz-aldehyde and HCl in the processed samples, the modern ultra-fast liquid chromatographic system with a very sensitive DAD equipped with a 10 μl flow cell assured excellent sensitivity, selectivity and precision of qualitative and quantitative determination of urea in all assayed samples by our new chromatographic procedure. Therefore, the presented ultra-fast chromatographic method (UFLC-DAD) offered significantly better sensitivity compared with our previous high-performance liquid chromatographic method with photodiode detection (HPLC-DAD) (Czauderna and Kowalczyk, 2009). Moreover, the proposed UFLC-DAD method provided slightly better accuracy and precision than our previous HPLC-DAD method. Altogether, the current chromatographic method (UFLC-DAD) and our previous HPLC-DAD method (Czauderna and Kowalczyk, 2009) assured better accuracy in comparison with other analytical methods (e.g. col-

orimetric methods, the specific Ureakvant method, enzymatic flow injection analysis and infrared methods) (Hanuš et al., 2008; Hering et al., 2008). Our chromatographic procedure is especially suitable for preparation of reference sample sets (e.g. in milk analysis instrument calibration), very accurate and precise research purposes (e.g. nutritional studies) or rapid clinical diagnostics with smaller sample sets. On the other hand, in comparison to our ultra-fast chromatographic method (UFLC-DAD) and our previous HPLC-DAD method, the Ureakvant procedure or infrared methods (especially with Fourier's transformations) are more effective routine methods for determination of urea in large number of samples of biological origin (e.g. milk samples) (Jílek et al., 2006; Hanuš et al., 2008; Hering et al., 2008; Řehák et al., 2009).

## CONCLUSION

Our ultra-fast liquid chromatography showed that more unidentified peaks appeared in the chromatograms of the processed biological materials when detection was performed at 254 nm instead of 370 nm. Indeed, chromatographic analyses of the assayed milk and plasma samples showed that the peak of derivatized urea monitored at 370 nm could be most suitably integrated, since this signal was devoid of the effect of substantial co-eluting impurities and endogenous species of significantly smaller absorptivity in the UV range of 350–400 nm than at 200–340 nm. Altogether, our ultra-fast chromatographic method with photodiode detection at 370 nm is a versatile analytical tool that assures simple, sensitive, accurate and precise analysis of urea in urine, milk and blood plasma samples as well as in selected diets of mammals. As urea is an indicator of liver and kidney function, its quantification in urine can be used by clinical laboratories. Moreover, our UFLC-DAD method used to quantify urea in urine can help to investigate the nutritional requirements of farm animals. Indeed, meat and milk production, reproductive performance, and nitrogen pollution of the environment depend upon the composition of rations for farm animals. Only detection of urea in urine at 254 nm is more sensitive and precise compared with monitoring at 370 nm. Thus, our chromatographic method-based detection at 254 nm and on widely available $C_{18}$ columns is suitable for simple non-invasive analysis of urea in urine samples of humans and animals. The proposed UFLC method with detection at 370 nm, based on relatively inexpensive and widely available $C_{18}$ columns, is particularly suitable for the analysis of urea in milk samples. Neither enzyme-related approach is satisfactory for selective and accurate quantification of the content of urea in milk samples due to the matrix effects (e.g. a variable content of fat).

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## REFERENCES

Carlsson J., Bergström J. (1994): The diurnal variation of urea in cow's milk and how milk fat content, storage and preservation affects analysis by a flow injection technique. Acta Veterinaria Scandinavica, 35, 67–77.

Clark S., Francis P.S., Conlan X.A., Barnett N.W. (2007): Determination of urea using high-performance liquid chromatography with fluorescence detection after automated derivatisation with xanthydrol. Journal of Chromatography A, 1161, 207–213.

Czauderna M., Kowalczyk J. (2001): Separation of some mono-, di- and tri-unsaturated fatty acids containing 18 carbon atoms by high-performance liquid chromatography and photodiode array detection. Journal of Chromatography B, 760, 165–178.

Czauderna M., Kowalczyk J. (2009): Easy and accurate determination of urea in milk, blood plasma, urine and selected diets of mammals by high-performance liquid chromatography with photodiode array detection preceded by pre-column derivatization. Chemical Analysis, 54, 919–937.

Dallet Ph., Labat L., Kummer E., Dubost J.P. (2000): Determination of urea, allantoin and lysine pyroglutamate in cosmetic samples by hydrophilic interaction chromatography. Journal of Chromatography B, 742, 447–452.

Dhawan G., Sumana G., Malhotra B.D. (2009): Recent developments in urea biosensors. Review. Biochemical Engineering Journal, 44, 42–52.

Ferguson J.D. (2000): Milk Urea Nitrogen. Center for Animal Health and Productivity, Pennsylvania, USA.

Francis P.S., Lewis S.W., Lim K.F. (2002): Analytical methodology for the determination of urea: current practice

and future trends. Trends in Analytical Chemistry, 21, 5, 389–400.

Gutiérrez M., Alegret S., del Valle M. (2008): Bioelectronic tongue for the simultaneous determination of urea, creatinine and alkaline ions in clinical samples. Biosensors and Bioelectronics, 23, 795–802.

Hanuš O., Hering P., Frelich J., Jilek M., Genčurova V., Jedelska R. (2008): Reliability of results of milk urea analysis by various methods using artificial milk control samples. Czech Journal of Animal Science, 53, 152–161.

Hering P., Hanuš O., Frelich J., Pytloun J., Macek A., Janů L., Kopecký J. (2008): Relationships between the results of various methods of urea analysis in native and enriched milk. Czech Journal of Animal Science, 53, 64–76.

Jílek F., Řehák D., Volek J., Štípková M., Němcová E., Fiedlerová M., Rajmon R., Švestková D. (2006): Effect of herd, parity, stage of lactation and milk yield on urea concentration in milk. Czech Journal of Animal Science, 51, 510–517.

Lin J.T., McKeon T.A. (2005): HPLC of Acyl Lipids. HNB Publishing, New York, USA.

Oltner R., Bengtsson S., Larson K. (1985): Flow injection analysis for the determination of urea in cows milk. Acta Veterinaria Scandinavica, 26, 396–404.

Petersom A.B., French K.R., Russek-Cohen E., Kohn R.A. (2004): Comparison of analytical methods and the influence of milk components on milk urea nitrogen recovery. Journal of Dairy Science, 87, 1747–1750.

Řehák D., Rajmon R., Kubešová M., Štípková M., Volek J., Jílek F. (2009): Relationships between milk urea and production and fertility traits in Holstein dairy herds in the Czech Republic. Czech Journal of Animal Science, 54, 193–200.

Sehitoğullari A., Uslan A.H. (2002): Preparation of a potentiometric immobilized urease electrode and urea determination in serum. Talanta, 57, 1039–1044.

Sejrsen K., Hvelplund T., Nielsen M.O. (2006): Ruminant Physiology. Wageningen Academic Publishers, Wageningen, the Netherlands.

Sheppard N.F., Mears D.J., Guiseppi-Elie A. (1996): Model of an immobilized enzyme conductimetric urea biosensor. Biosensors and Bioelectronics, 11, 967–979.

Yatzidis H., Garidi M., Vassilikos C., Mayopoulou D., Akilas A. (1964): An improved method for the simple and accurate colometric determination of urea with Ehrlich's reagent. Journal of Clinical Pathology, 17, 163–164.

Zhai S.W., Liu J.X., Wu Y.M., Ye J.A., Xu Y.N. (2006): Responses of milk urea nitrogen content to dietary crude protein level and degradability in lactating Holstein dairy cows. Czech Journal of Animal Science, 51, 518–522.

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