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Determination of uric acid in urine, saliva and calcium oxalate renal calculi by high-performance liquid chromatography/mass spectrometry

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Abstract

A very simple and direct method for determination of uric acid, in various biological matrices, based on high-performance liquid chromatography and mass spectrometry is described. Chromatographic separations were performed with a stationary phase Zorbax Sax Column, an anion exchange resin, with 50% sodium citrate 1 mM at pH 6.5 and 50% acetonitrile as mobile phase delivered at a flow rate of 1 ml/min. The detector counted negative ions by monitoring *m*/*z* 167.1, which corresponds to the urate anion. The method does not use an internal standard but quality control samples were used. Intra-day precision ranged between 1.1 and 1.5%, whereas inter-day precision was between 1.3 and 2.8% ($n = 5$) working with some selected standards. Recovery tests of added standard have been successfully performed in urine and saliva samples, thus showing an appropriate accuracy of the method. The limit of quantitation found was 70 μg/l. Different urine and saliva samples were analyzed using an alternative analytical methodology based on an enzymatic reaction and photometric detection at 520 nm, resulting both methods comparable at a 95% confidence level. The method has been also applied to the determination of trace amounts of uric acid in the core of some selected calcium oxalate renal calculi.

Keywords: Uric acid; Liquid chromatography; Mass spectrometry; Renal calculi

1. Introduction

Uric acid (2,6,8-trihydroxypurine, UA) is the major nitrogenous compound in urine, but it is also found in other biological fluids such as serum, blood and saliva. UA is the main final product of purine metabolism [1,2], and its determination in urine is a powerful indicator of metabolic alterations or disease appearance [3]. Likewise, excessive production of UA may lead to its precipitation in the kidney at low pH values [4]. Around 8% of renal calculi are formed by UA [5] and there are two main causes to explain this undesiderable crystallization: the supersaturation of urine with UA [6] and low pH values (<5.5). It has also been demonstrated that a group of calcium oxalate monohydrate renal calculi have a core formed by important amounts of UA, which acts as heterogeneous nucleant [5]. In some cases due to the little size of the core or due to the presence of abundant organic matter, the presence of uric acid can not be confirmed, in spite of its importance to stablish the calculus ethiology and as a consequence the appropriate pharmacologyc and/or dietetic treatment [5]. Furthermore, one of the biggest problems about the UA metabolism is Gout [4], which can be caused by an increase in UA production and a decrease in elimination of UA by the kidneys, or by an increase of intake of foods containing purines (which are also metabolized to UA). Elevated levels of UA can be caused by many factors, including increased alcohol consumption, obesity, diabetes, high cholesterol, high blood pressure, kidney disease, and heart disease. UA has been reported to act as an antioxidant

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[7]. As a consequence, determination of UA for diagnosis and treatment of various disorders is very important.

The concentration of UA in urine is about 250–750 mg/l for healthy adult people [8].

Analytical methods for the determination of UA can be classified into four main groups: colorimetric methods, enzymatic methods, high-performance liquid chromatographic (HPLC) methods and methods based on the use of biosensors. The first analytical method described to determine UA dates from 1894, when Offer used the antioxidant properties of UA to determine it via the reduction of phosphotungstate complexes and colorimetric detection [9]. In 1895 other methods appeared with the same principle, but all of them presented numerous stages of sample pre-treatment.

The first enzymatic methods appeared in 1941, when UA was determined employing the enzime uricase [10] and performing absorbance measurements at 293 nm. Other methods have been described using the same principles, but their main drawback is the need of protein disruption for each sample. Using the same reaction, H_2O_2 generated can be used, in the presence of peroxidase, to oxidize a cromogenic dye, originating a red complex that absorbs at 520 nm [11–14]. Clinical laboratories use this sequence of reactions as routinary method for UA determination [8].

However, chemical methods give higher values due to the presence of endogenous and exogenous substances that reduce phosphotungstate [15]. Enzymatic assays (in spite of being very selective) still suffer from interference by various substances (such as metals) that lead to negative errors. To avoid these interferences, many HPLC methods were developed. Ion-exchange [16–18], ion-pair [19,20], reversed phase [21,22] and size-exclusion chromatography [23] were used for UA determination. The main problem of most of these methods is the need of deproteination of serum samples before analysis. This can be achieved following either the procedure of Somogyi [24] or the procedure of Haden [25]. It can also be performed by adding trichloroacetic acid, perchlorate or acetonitrile [15], but in all cases samples need centrifugation and further treatment with a phosphate buffer. Also, ultrafiltration has been used to perform deproteination.

A recent method for the determination of UA in human saliva using electrochemical detection has been developed [26].

Also, several methods have been developed for UA determination using biosensors [27–32]. These methods are based on the enzymatic reaction or similar reactions described above. In this case, an enzyme suspension is immobilized on the surface of a membrane.

In a recent paper [33], several organic acids have been determined in urine by ion-pair chromatography and capillary zone electrophoresis, with detection limits of 0.11 and 0.87 mg/l UA, respectively.

In this paper, a very simple, sensitive and selective (due to the characteristics of the detection) HPLC–MS methodology for UA determination is described and applied to real urine and calcium oxalte renal calculi.

2. Experimental

2.1. Quantitation method

Analysis of UA was performed with an Agilent 1100 Series HPLC–MS system. Chromatographic separations were performed at 25 °C on a Zorbax Sax Column (150 mm × 4.6 mm i.d.), an anion exchange resin, 5 μm particle size (supplied by Agilent Technologies, Madrid, Spain) with a 12.5 mm × 4.6 mm i.d. guard column (supplied by Agilent Technologies, Madrid, Spain). The mobile phase (50% sodium citrate 1 mM at pH 6.5, 50% acetonitrile) was delivered at a flow rate of 1 ml/min. Mass spectral identification of UA was carried out with an electrospray ionization interface and a quadrupole mass analyzer. The mobile phase was nebulized by nitrogen gas at 350 °C, with a flow rate of 13 l/min, into an electrospray mass analyzer. The detector counted negative ions with selected ion monitor (SIM) mode, by monitoring *m/z* 167.1 Th, which corresponds to the urate anion, the most abundant ion. The nebulization pressure used was 60 psi and the fragmentor voltage 80 V. Capillary voltage was 3000 V. As no internal standard was used, quality control samples were used in all analysis to monitor possible deviations of the instrument.

2.2. Reagents

All chemicals were of analytical-reagent grade. UA was purchased from Fluka (CH, Switzerland), sodium citrate and acetonitrile from Panreac (Barcelona, Spain), methanol and tetrahydrofuran from Scharlau (Barcelona, Spain). Solutions were prepared with Milli-Q (18.2 MΩ cm) distilled-deionised water and filtered through 0.45 μm pore filters from Sugelabor S.A. (Madrid, Spain).

2.3. Sample treatment

2.3.1. Treatment for urine

Urine samples containing UA within the 250–750 mg/l range were 100-fold diluted with Milli-Q water and the resultant solution was filtered through 0.45 μm pore filters.

2.3.2. Treatment for saliva

Saliva samples containing UA within the 5–30 mg/l range were 20-fold diluted with Milli-Q water and the resultant solution was filtered through 0.45 μm pore filters.

2.3.3. Treatment for renal calculi

The core of some selected cavity calcium oxalate monohydrate renal calculi was detached, pulverized and uric acid extracted with 5 ml of NaOH at pH 11 and the extracts were filtered through 0.45 μm pore filters.

In all cases, 10 μl of the solution were injected in the HPLC–MS system.

Standards were prepared from aqueous solutions of UA and the analytical determination was carried out using the corresponding calibration curve.

3. Results and discussion

Most of the described methods for UA determination suffer from interferences coming from other substances present in the matrix of the sample. Due to the characteristics of mass detection, high selectivity is obtained in UA determination. Also, the high sensitivity of the detection allows a 100- and 20-fold dilution of urine and saliva samples, respectively, which avoids matrix effects (as is demonstrated with tests of standard addition) and sample pre-treatment (see Fig. 1a). Furthermore, the high sensitivity of the proposed methodology (LOD = 0.21 ng UA) allows the quantification of trace amounts of UA in the core of calcium oxalate monohydrate renal calculi (see Table 4).

All these reasons make this methodology one of the simplest described for UA determination.

Negative ion mode was selected due to the higher sensitivity. In the positive ion mode, the most abundant peaks in the mass spectrum were 169.1 and 191.1, that corresponded to UA adducts with H^+ and Na^+, respectively. Although sensitivity was sufficient to quantify UA present in urine and saliva samples, the negative ion mode would permit further UA determination in other samples, such as trace amounts of UA in the core of cavity calcium oxalate monohydrate renal calculi.

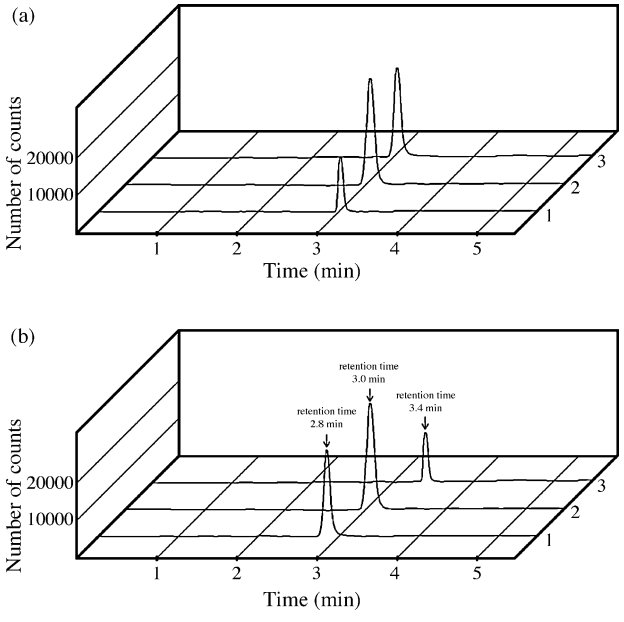

Fig. 1. (a) SIM chromatograms obtained by injection of 10 μl of (1) standard solution of 2.5 mg/l UA; (2) standard solution of 5 mg/l UA and (3) urine sample containing 420.5 mg/l UA and working as described in Section 2. Mobile phase: 50% sodium citrate 1 mM at pH 6.5, 50% acetonitrile. (b) SIM chromatograms obtained by injection of 10 μl of a standard solution of 5 mg/l UA and working as described in Section 2. Mobile phases assayed: (1) 50% sodium citrate 1 mM at pH 6.5, 50% tetrahydrofuran; (2) 50% sodium citrate 1 mM at pH 6.5, 50% acetonitrile and (3) 50% sodium citrate 1 mM at pH 6.5, 50% methanol.

3.1. Study of variables

3.1.1. Mobile phase composition

Sodium citrate in aqueous solution was selected as mobile phase because it allowed a successful separation and was suitable for mass detection.

Three different organic solvents were tested as organic modifiers (tetrahydrofuran, acetonitrile and methanol). As can be seen in Fig. 1b, retention times were similar in all cases, but a higher sensitivity was obtained with acetonitrile.

Then, different acetonitrile proportions were studied to perform the separation. A higher sensitivity was obtained using 50% of acetonitrile and a decrease of analytical signal was observed at higher organic percentages.

Working under such conditions, joint to SIM mode, no peaks apart from UA were found during the first 5 min of analysis with the three types of assayed samples (urine, saliva and renal calculi), thus demonstrating the specificity of the method.

3.1.2. Instrumental variables

Five variables corresponding to the mass detector were optimized in order to improve sensitivity.

The fragmentor voltage was the most critical variable because at low voltages, adducts formed in the mobile phase could not be fragmented, whereas at high voltage values, the analyte was fragmented and decreased the relative abundance of the molecular ion.

Drying gas flow, nebulization pressure, gas temperature and capillary voltage were also optimized. Conditions of maxium sensibility (described in the material and methods section) were selected in all cases except for capillary voltage, where 3000 V were chosen to lengthen mass detector lifetime.

3.2. Characteristics of the analytical method and application

3.2.1. Linearity

There was a linear relationship between detector response and amount of UA over a range of 0.7–100 ng of UA (0.07–10 mg/l, taking into account the sample volume injected).

3.2.2. Limit of detection

The limit of detection of UA (calculated as $3\,s_{y/x}$/sensitivity) was 0.21 ng, while the limit of quantification (calculated as $10\,s_{y/x}$/sensitivity) was 0.70 ng. Thus, taking into account the injected volume (10 μl) the limit of detection and the limit of quantification would be, respectively, 21 and 70 μg/l, corresponding to the injected solution. These are

Table 1
Study of the accuracy of the proposed methodology in urine samples

Sample	Uric acid		
	Added (mg/l)	Found (mg/l)	Recovery (%)
Urine 1	–	667.4	–
	25	691.7	97.2
Urine 2	–	292.0	–
	25	318.8	107.2
Urine 3	–	132.1	–
	25	157.6	102.0
Urine 4	–	346.4	–
	25	370.4	96.0
Urine 5	–	279.3	–
	25	303.5	96.8
Urine 6	–	153.5	–
	25	179.2	102.8
Urine 7	–	420.5	–
	25	444.3	95.2
Urine 8	–	256.5	–
	25	282.8	105.2
Urine 9	–	247.5	–
	25	271.5	96.0
Urine 10	–	253.5	–
	25	277.6	96.4
Urine 11	–	340.5	–
	25	364.7	96.8

Table 2
Study of the accuracy of the proposed methodology in saliva samples

Sample	Uric acid		
	Added (mg/l)	Found (mg/l)	Recovery (%)
Saliva 1	–	16.3	
	3	19.4	103.4
	6	22.3	100.1
	10	26.8	105.3
Saliva 2	–	29.8	
	3	32.7	98.6
	6	36.0	103.8
	10	40.1	103.7
Saliva 3	–	25.5	
	3	28.8	108.2
	6	31.4	98.2
	10	35.4	98.4
Saliva 4	–	16.2	
	3	19.4	103.8
	6	22.7	106.8
	10	25.9	96.4
Saliva 5	–	29.2	
	3	32.2	99.3
	6	35.5	105.8
	10	40.1	109.2
Saliva 6	–	26.8	
	3	29.9	104.2
	6	33.2	107.0
	10	37.4	106.9
Saliva 7	–	13.7	
	3	16.8	102.5
	6	19.5	96.2
	10	24.1	103.5
Saliva 8	–	26.8	
	3	29.6	95.8
	6	32.5	95.4
	10	36.3	95.2

very reduced amounts compared with those normally found in urine and saliva samples and they permit an important sample dilution that avoids matrix effects; moreover, the high sensitivity supplies an analytical methodology which is unique for the quantification of UA that acts as heterogeneous nucleant in the core of cavity calcium oxalate monohydrate renal calculi, and this fact could have important implications in the diagnosis and treatment of these patients.

3.2.3. Accuracy and precision

The proposed HPLC–MS method has been used for the analysis of several urine and saliva samples, and recovery tests of added standard have been carried out to test the accuracy of the method. Results are summarized in Tables 1 and 2. The regression line *UA in natural samples* (Y) versus *UA found in spiked samples* (X, *corrected for the UA added in the spike*) was compared with the theoric line $Y = X$. Slope and intercept of the regression lines obtained with urine ($Y = (1.003 \pm 0.002)X + (-0.694 \pm 0.762)$ ($s_{y/x} = 1.031$, $n = 11$, $R^2 = 0.999$)) and saliva samples ($Y = (1.014 \pm 0.031)X + (-0.081 \pm 0.746)$ ($s_{y/x} = 0.539$, $n = 8$, $R^2 = 0.994$)) were statistically comparable (at a 95% confidence level) to 1 and 0, respectively, thus demonstrating that standard addition is not needed for UA quantification in urine and saliva samples.

Following, the method was also validated at a lower concentration range, working with renal calculi samples. As can be seen in Fig. 2, different calibration lines were obtained working with standards and with samples to which different amounts of UA standard were added. In all cases, the slopes obtained working with sample matrices were statisti-

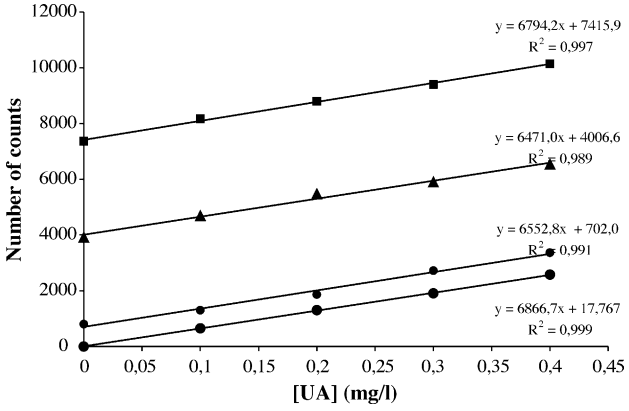

Fig. 2. Study of standard addition to renal calculi sample matrices.

cally comparable at a 95% confidence level with the slope obtained working with UA standards.

The relative standard deviations of some selected standards (0.5–1 mg/l UA) ranged between 1.1 and 1.5% ($n = 5$) when analyzed on the same day, and between 1.3 and 2.8% ($n = 5$) when analyzed at different days, thus showing a good repeatability and reproducibility.

The precision of the methodology was also studied at two different levels of UA concentration, working with renal calculi, urine and saliva samples, respectively. The relative standard deviations of five different renal calculi samples with concentrations in the 0.18–0.27 mg/l range oscillated between 0.9 and 1.2% ($n = 5$) when analyzed on the same day, and between 1.2 and 3.0% ($n = 5$) when analyzed at different days. The values for urine samples with an injected concentration ranging from 1.5 to 8.1 mg/l UA were 1.0–1.9% for intra-day precision ($n = 5$) and 1.5–2.7% ($n = 5$) for inter-day precision. With saliva samples, the intra-day precision ($n = 5$) ranged from 1.3 to 1.8% and the inter-day precision ($n = 5$) between 1.7 and 3.0%.

3.2.4. Comparison with an alternative analytical procedure

Several samples were also analysed according to an alternative procedure, based upon the methods of Trivedi et al. [12] and Kabasakalian et al. [13] with a modified Trinder [14] peroxide assay using 2,4,6-tribromo-2-hydroxy benzoic acid. The method is based on the enzymatic conversion of UA into allantoin with the production of H_2O_2. The peroxide reacts with 4-aminoantipyrine and 2,4,6-tribromo-3-hydroxy benzoic acid in the presence of peroxidase to yield a quinoneimine dye. The resulting change in absorbance at 520 nm is proportional to UA concentration in the sample. The results of these determinations are shown in Table 3. Both methods were comparable with a 95% confidence level. In this way, the obtained regression graphs for 18 urine samples ($y = 1.019x + 10.912$, $R^2 = 0.992$) and 8 saliva samples ($y = 0.969x + 0.923$, $R^2 = 0.967$) (where y represents obtained concentration by the present method and x concentration by the enzymatic-photometric) were statistically comparable to the graph $Y = X$ at a 95% confidence level, thus showing a good agreement between the two methods and demonstrating the suitability of the proposed procedure. It must be taken into account that the comparison of both methodologies has been performed using urine and saliva samples due to the lower sensibility of the photometric method that could not allow UA determination in renal calculi.

Table 3
UA content (mg/l ± %CV) of human urine and saliva samples applying two different analytical methods ($n = 3$)

Sample	Method enzymatic-photometric [10]	Method HPLC–MS (this paper)
Urine 1	633 ± 20	637 ± 8
Urine 2	359 ± 12	406 ± 6
Urine 3	758 ± 18	812 ± 11
Urine 4	532 ± 15	569 ± 8
Urine 5	595 ± 13	619 ± 9
Urine 6	157 ± 8	162 ± 3
Urine 7	418 ± 12	429 ± 5
Urine 8	401 ± 8	406 ± 4
Urine 9	631 ± 22	642 ± 8
Urine 10	575 ± 15	581 ± 6
Urine 11	426 ± 15	426 ± 7
Urine 12	294 ± 10	300 ± 4
Urine 13	705 ± 18	717 ± 8
Urine 14	327 ± 12	350 ± 4
Urine 15	398 ± 11	429 ± 5
Urine 16	134 ± 10	150 ± 2
Urine 17	740 ± 16	753 ± 9
Urine 18	549 ± 16	603 ± 9
Saliva 1	16.6 ± 0.9	16.3 ± 0.2
Saliva 2	31.2 ± 1.2	29.8 ± 0.3
Saliva 3	24.3 ± 1.3	25.5 ± 0.3
Saliva 4	16.9 ± 1.0	16.2 ± 0.2
Saliva 5	28.6 ± 1.5	29.2 ± 0.3
Saliva 6	27.5 ± 1.5	26.8 ± 0.3
Saliva 7	12.8 ± 1.4	13.7 ± 0.2
Saliva 8	24.6 ± 1.4	26.8 ± 0.2

Both methods were comparable with a 95% confidence level.

3.2.5. Application to calcium oxalate renal calculi

The proposed analytical methodology has been applied to the determination of UA in the core of two different types of calcium oxalate renal calculi. Five of them, which were cavity calcium oxalate monohydrate renal calculi, had UA as important component in the core. Seven of them were calcium oxalate monohydrate renal calculi with a very little size core formed by unidentified organic matter. In these latter (see Table 4) UA would be unquantificable with conventional

Table 4
UA content (mg UA/g core ± %CV) in the core of some selected calcium oxalate monohydrate renal calculi ($n = 3$)

Calcium oxalate renal calculi with a core formed by UA as an important component			Calcium oxalate renal calculi with a very little size core formed by unidentified organic matter		
Sample number	mg UA/g core ± % CV	Sample weight (mg)	Sample number	mg UA/g core ± % CV	Sample weight (mg)
1	113 ± 2	1.3	1′	0.18 ± 0.01	2.9
2	40 ± 1	0.5	2′	6.21 ± 0.08	0.9
3	284 ± 3	0.6	3′	7.49 ± 0.08	0.3
4	818 ± 11	0.1	4′	0.80 ± 0.01	1.9
5	27 ± 1	2	5′	3.28 ± 0.03	1.3
			6′	0.042 ± 0.001	9.7
			7′	0.69 ± 0.01	1.7

photometric and enzymatic methodologies [12–14,34–36]. Even some recent methods have limits of detection in the 0.1–1 mg/l UA range [33,37,38].

Obviously, in the case of renal calculi there are no matrix effects because at pH 11 calcium oxalate is insoluble and only UA is extracted. The results of these analysis are shown in Table 4.

3.2.6. Quality control samples

As no internal standard was used, the method was monitored using quality control samples. Method blanks, replicates, duplicates, knowns and spikes were used.

Method blanks were introduced and were considered as acceptable if they were inferior to the detection limit.

Replicates and duplicates were considered acceptable if precision did not exceed 2 and 3%, respectively.

Knowns were accepted as correct if their accuracy was inferior to 5% and in spikes, recoveries ranging from 90 to 110% were considered as acceptable.

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References

[1] E. Popa, Y. Kubota, D.A. Tryk, A. Fujishima, Anal. Chem. 72 (2000) 1724.
[2] G. Dryhurst, Electrochemistry of Biological Molecules, Academic Press, New York, 1977.
[3] V.S.E. Dutt, H.A. Mottola, Anal. Chem. 46 (1974) 1777.
[4] P. Ellerbe, A. Cohen, M.J. Welch, E.V. White, Anal. Chem. 62 (1990) 2173.
[5] F. Grases, A. Costa-Bauzá, M. Ramis, V. Montesinos, A. Conte, Clin. Chim. Acta 322 (2002) 29.
[6] B. Finlayson, L. Bubois, Clin. Chem. 84 (1978) 203.
[7] O. Lux, D. Naidoo, C. Salonikas, Ann. Clin. Biochem. 29 (1992) 674.
[8] R.J. Henry, D.C. Cannon, J.W. Winkelman, Clinical Chemistry Principles and Technics, second ed., Harper & Row, Hagerstown, 1974.
[9] T.R. Offer, Centr. Physiol. 8 (1894) 801, apud [10].
[10] H.A. Bulger, H.E. Johns, J. Biol. Chem. 140 (1941) 427.
[11] K. Lorentz, W. Berndt, Anal. Biochem. 18 (1967) 58.
[12] R. Trivedi, E. Berta, L. Rebar, Clin. Chem. 22 (1976) 1223.
[13] P. Kabasakalian, S. Kalliney, A. Wescott, Clin. Chem. 19 (1973) 522.
[14] P. Trinder, J. Clin. Pathol. 22 (1949) 246.
[15] R. Sakuma, T. Nishina, M. Kitamura, Clin. Chem. 33 (1987) 1427.
[16] J.A. Milner, E.G. Perkins, Anal. Biochem. 88 (1978) 560.
[17] T. Dülffer, E. Hägele, M.H. Town, J. Ziegenhorn, Z. Anal. Chem. 324 (1986) 333.
[18] T. Etoh, M. Iwatake, M. Miyazaki, K. Harada, M. Nakayama, A. Sugii, Y. Uji, H. Okabe, J. Liq. Chromatogr. 15 (1992) 1656.
[19] H. Müller, Z. Anal. Chem. 332 (1988) 464.
[20] M.A. Ross, J. Chromatogr. B 657 (1994) 197.
[21] T. Kojima, T. Nishina, M. Kitamura, N. Kamatani, K. Nishioka, Clin. Chem. 32 (1986) 287.
[22] R. Sakuma, T. Nishina, M. Kitamura, Clin. Chem. 33 (1987) 1427.
[23] M. Yoshiura, K. Iriyama, J. Liq. Chromatogr. 9 (1986) 177.
[24] M. Somogyi, J. Biol. Chem. 32 (1945) 69.
[25] R.L. Haden, J. Biol. Chem. 56 (1923) 469.
[26] K. Inoue, T. Namiki, Y. Iwasaki, Y. Yoshimura, H. Nakazawa, J. Chromatogr. B 785 (2003) 57.
[27] Y.Q. Zhang, W.D. Shen, R.A. Gu, J. Zhu, R.Y. Xue, Anal. Chim. Acta 369 (1998) 123.
[28] M.A.T. Gilmartin, J.P. Hart, Analyst 119 (1994) 833.
[29] S. Uchiyama, S. Suzuki, T. Sato, Electroanalysis 2 (1990) 559.
[30] F.H. Keedy, P. Vadgama, Biosens. Bioelectron. 6 (1991) 491.
[31] J. Motonaka, K. Miyata, L.R. Faulkner, Anal. Lett. 27 (1994) 1.
[32] E. Miland, A.J.M. Ordieres, P.T. Blanco, M.R. Smyth, C.O. Fagain, Talanta 43 (1996) 785.
[33] W. Shu-Ping, L. Chiou-Shyi, J. Chromatogr. A 1051 (2004) 213.
[34] Z. Gong, Z. Zhang, Anal. Lett. 29 (1996) 695.
[35] R.R. Marquardt, A.T. Ward, L.D. Campbell, Poult. Sci. 62 (1983) 2099.
[36] S.D. Yang, J.M. Xu, L. Yang, Y.N. Ma, F. Bai, J. Liq. Chromatogr. 12 (1989) 1791.
[37] H.H. Cho, Z.U. Bae, Anal. Sci. 17 (2001) a89.
[38] M. Jelikic-Stankov, P. Djurdjevic, D. Stankov, J. Serbian Chem. Soc. 68 (2003) 691.