Journal of Chromatography, 568 (1991) 105–115
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5893

Quantitation of hypoxanthine in plasma from patients with ischemic heart disease: adaption of a high-performance liquid chromatographic method

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(First received October 1st, 1990; revised manuscript received March 5th, 1991)

ABSTRACT

A high-performance liquid chromatographic method is described for the separation and quantitation of several purine compounds, including hypoxanthine. The isocratic separation of a standard mixture of nine compounds is achieved within 20 min on a reversed-phase Nucleosil 100-5C_{18} column, with a mobile phase of KH_2PO_4 (300 m*M*, pH 4.0)–methanol–acetonitrile–tetrahydrofuran (97.9:1:1:0.1, v/v). Uric acid, guanine, hypoxanthine, uridine, xanthine, allopurinol, inosine, guanosine and 7-methylxanthine were almost completely baseline-separated, with detection limits in the range 0.5–1.2 pmol per injection. The influence of the concentrations of buffer and tetrahydrofuran on the quality of separation are described. The within-day and the day-to-day precision were satisfactory (*e.g.* coefficients of variation of less than 1.5 and *ca.* 6.0%, respectively, for peak heights). The recovery of [^{3}H]hypoxanthine added to samples was 86 ± 1%. Hypoxanthine was quantified in human plasma samples obtained at various times during coronary artery bypass grafting. The hypoxanthine levels measured immediately after release of the aortic cross-clamp were significantly higher than those determined under control conditions (18.8 ± 7.0 and 3.4 ± 1.0 μM, respectively).

INTRODUCTION

During severe myocardial ischemia, the stable metabolite of ATP, hypoxanthine, appears in the extracellular and vascular space. Therefore, hypoxanthine has been used as a marker for the degree of ischemic damage [1–3], and was found to be a more sensitive indicator of ischemia than lactate accumulation or even clinical signs, such as angina or ST-segment elevation [4,5].

The use of hypoxanthine for the follow-up of the energy status of the myocar-

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dium requires a precise and sensitive method of analysis. Furthermore, in clinical routine, the method should be as rapid and inexpensive as possible. In this regard, high-performance liquid chromatography (HPLC) is the method of choice (for recent review see ref.1). However, the HPLC methods reported so far have drawbacks, such as (i) insufficient separation of hypoxanthine from other purine compounds [6–10], (ii) lengthy analysis (use of gradient elutions methods shortens the analysis time, but this effect is offset by the long periods needed for reequilibration of the column [10–14]), (iii) use of high concentrations of toxic eluents, such as acetonitrile [15], and (iv) use of rather expensive column packing materials, such as μBondapak C_{18} [2,4,5,8,11–13,15–19], Hypersil ODS [6,20–22] or RP Select B [23].

This paper describes an adaption of an HPLC method with isocratic elution for the determination of hypoxanthine. We have developed this method to meet our requirements for the detection of a wide range of purine compounds, and for good separation within reasonable time and at reasonable cost. The method has been tested in a clinical application.

EXPERIMENTAL

Chemicals and chromatographic standards

Purine compounds and other standards were obtained from Sigma (Munich, Germany). Trioctylamine, methanol, Freon® and trichloroacetic acid (TCA) were from Baker (Deventer, Netherlands); the highest purity available was used unless otherwise stated. All other chemicals were from Merck (Darmstadt, Germany). Demineralized, distilled water (Milli-Q, Millipore, Eschborn, Germany) was used throughout. Xanthine oxidase (EC 1.2.3.2) used for peak identification was from Sigma. [G-^{3}H]Hypoxanthine (specific activity 5.8 Ci/mmol) used in recovery experiments was from Amersham (Braunschweig, Germany).

Chromatographic equipment

The chromatographic system consisted of a Marathon autosampler (Spark, Emmen, Netherlands), a Model 114M pump (Beckman, Munich, Germany), and a single-wavelength absorbance detector set at either 254 nm or 280 nm (Model 166, Beckman). System control and data acquisition, storage and evaluation were done with the software System Gold® (Beckman).

Stainless-steel columns (250 mm × 4.6 mm I.D.) packed with either Ultrasphere ODS 5 μm (Beckman) or Kromasil 5C_{18} or Nucleosil 100-5C_{18} (both CS Chromatographie Service, Langerwehe, Germany) were used for chromatography. The analytical column was usually protected by a precolumn (50 mm × 4.6 mm I.D.) packed with Spherisorb C_{18} (Beckman).

Chromatographic conditions

The isocratic elution was performed with potassium phosphate buffers of vary-

ing ionic strength and pH; the amount and composition of the organic part of the eluent were also varied. The final eluent was KH_2PO_4 (300 m*M*, adjusted to pH 4.0 with 1 *M* phosphoric acid)–methanol–acetonitrile–tetrahydrofuran (97.9:1:1:0.1, v/v). Separation was performed at a column temperature of 30°C and a flow-rate of 1.0 ml/min, resulting in a pressure of *ca.* 160 bar. After filtration through GVWP membrane filters (Millipore, Eschborn, Germany), the mobile phase was degassed by continuously bubbling with helium.

Preparation and handling of samples and standards

Blood samples (peripheral venous, coronary venous and arterial) were obtained from eight patients undergoing coronary artery bypass grafting. The samples were collected into EDTA vials and immediately centrifuged (3000 *g*, 10 min, 4°C) to prevent contamination by endogenous formation of hypoxanthine in erythrocytes [10,20]. Aliquots (1 ml) of the separated plasma were stored in 1.5-ml Eppendorf vials at −20°C until analysis.

Plasma was handled as follows. A sample of 450 μl of plasma or water was spiked with 50 μl of a standard stock solution of an appropriate concentration. The plasma proteins were separated by precipitation with TCA (1 ml, 6%, w/v), vigorous mixing and centrifugation for 2 min in a Model 3200 microcentrifuge (Eppendorf) at maximum speed. A 750-μl volume of trioctylamine (0.5 *M*) in Freon was added to 750 μl of the supernatant; this was followed by vigorous mixing and centrifugation for 2 min; 500 μl of the supernatant were placed into special glass vials 11-AC3 (Chromacol, London, UK) to be handled by the autosampler of our HPLC system. (In later experiments conical plastic vials P-03 (CS Chromatographie Service) filled with 200 μl of the supernatant were used.) The injection volume was either 50 or 20 μl.

For peak identification and quantification, working standards were prepared by appropriate mixing and serial dilution of stock standard solutions to cover the following concentration ranges (in pmol per injection): 0–20 for hypoxanthine, guanine, xanthine, uridine and allopurinol; 0–200 for uric acid. The working standards were stored at −20°C; a new set of standards was thawed for each series of analyses and kept on ice until injection. Standards were prepared as aqueous stock solutions of guanine, guanosine, inosine, hypoxanthine, xanthine, uridine (300 μ*M* each) and uric acid (3 m*M*). Stock solutions of 7-methylxanthine and allopurinol used as an external standards were 300 μ*M*. Aliquots of the standard stock solutions were stored in the same way as human plasma.

The samples for the determination of the recovery of hypoxanthine were prepared from 440 μl of plasma, 10 μl of a solution of hypoxanthine (50 μ*M*), and 50 μl of a solution of [^{3}H]hypoxanthine (final concentration 50 n*M*, yielding *ca.* 6000 dpm). Protein precipitation with TCA and trioctylamine–Freon extraction were performed as described above. Ultrafree-MC 10K NMWL vials (Millipore) were used for removal of plasma proteins by ultrafiltration were mixed with 2 ml of a scintillation cocktail (Ultima Gold, Hewlett-Packard). The radioactivity was determined for a maximum of 10 min in a Beckman counter.

Quantitation of compounds and evaluation of data

Calibration curves for the standard compounds were established with six concentration levels covering the range 0–20 pmol per injection (*i.e.* 0–10 μM; hypoxanthine, xanthine, uridine, guanine, allopurinol) and 0–200 pmol per injection (*i.e.* 0–100 μM; uric acid), respectively. The least-squares linear regressions yielded correlation coefficients of $r > 0.99$ for all compounds. All measurements were performed at least in duplicate. The concentrations of compounds were calculated using the peak heights. The data are expressed as mean values ± S.D., or as mean values and the relative coefficient of variation (C.V.). The number of experiments is given in brackets. Student's *t*-test was used to evaluate statistical differences, which are considered significant if $p < 0.05$ (in ref. 24).

RESULTS AND DISCUSSION

Basic method

The separation of purine compounds by reversed-phase HPLC is often accomplished by means of mobile phases containing phosphate buffer (1–100 mM) and methanol (1–60%, v/v) in either isocratic or gradient elutions (for reviews see refs. 1 and 25). Although gradient elution is favourable in terms of peak shape and time per run, one major disadvantage is the need for long re-equilibration times, especially when using extremely high detector sensitivities. Therefore, we decided to use an isocratic elution method resembling previous approaches [6,7,19,22,26].

With an Ultrasphere ODS 5-μm column at room temperature and a mobile phase of KH_2PO_4 (20 mM, pH 4.3)–methanol (97:3, v/v), baseline separation of hypoxanthine, xanthine and uric acid was rapid after the injection of an aqueous standard solution (see inset of Fig. 1A). However, unsatisfactory separation was obtained with human plasma samples, since hypoxanthine, the compound of major interest, was only poorly resolved from a triangular tailing peak, which presumably was caused by several compounds, including xanthine (see Fig. 1A). In order to compare peak separation and relative duration of analysis under various chromatographic conditions, the capacity factors k' were calculated [$k' = (t_r - t_0)/t_0$, with retention time t_R and dead time t_0]. Their logarithms are shown diagramatically in Fig. 1B.

Variations of ionic strength and pH did not significantly increase the quality of separation of uric acid, guanine, hypoxanthine, xanthine, inosine and guanosine. It was concluded that the chromatographic conditions chosen were not suitable for the separation of guanine, hypoxanthine and xanthine. We therefore changed the stationary phase to Kromasil $5C_{18}$, a packing material claimed to have similar properties (information by CS Chromatographie Service) to RP Select B, which yielded an excellent separation in the hands of Wynants *et al.* [23]. However, neither this change nor the addition of acetonitrile to the mobile phase resulted in any significant improvement (data not shown).

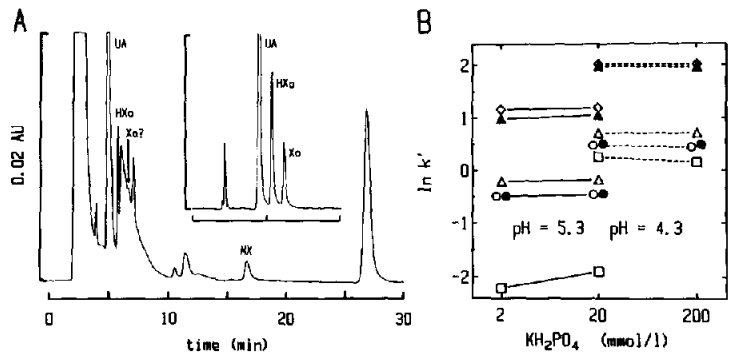

Fig. 1. (A) Chromatograms of human plasma (after protein precipitation with TCA and extraction with trioctylamine–Freon) and of aqueous standard (inset). Peaks: UA = uric acid; HXa = hypoxanthine; Xa = xanthine; MX = 7-methylxanthine; other peaks unidentified. Conditions: column, Ultrasphere ODS, 5 μm; mobile phase, KH_2PO_4 20 m*M* (pH 4.3)–methanol (97:3 v/v); injection volume, 50 μl; flow-rate, 1.0 ml/min; room temperature; detection wavelength, 254 nm. Calibration (for both chromatograms): absorbance, 0.02 a.u.f.s. (B) Influence on capacity factors *k'* (ordinate: ln *k'*) of variations of ionic strength (abscissa), pH and methanol concentration. Left side (2 and 20 m*M* of KH_2PO_4, solid lines): pH 5.3 and 3% methanol (v/v); right side (20 and 100 m*M* of KH_2PO_4, dashed lines): pH 4.3 and 1% methanol (v/v). Conditions: column, Ultrasphere ODS, 5 μm; injection volume, 50 μl; flow-rate, 1.0 ml/min; room temperature; detection wavelength, 254 nm. Symbols: (□) uric acid; (○) guanine; (●) hypoxanthine; (△) xanthine; (▲) inosine; (◆) guanosine.

In a search for better conditions, we tried using Nucleosil 100-5C_{18} as the packing material. Nucleosil columns are suitable for a wide range of purposes, and have been used for the separation of purine compounds. However, in one case gradient elution was necessary [14], and in the other the separation of hypoxanthine and xanthine was not satisfactory [9]. As an eluent we chose KH_2PO_4 (300 m*M*, pH 4.0)–methanol–acetonitrile–THF (97.9:1:1:0.1, v/v), as adapted from ref. 23. Under these conditions, the separation of a standard mixture of nine compounds was complete within 20 min, and almost all peaks were baseline separated (for a representative chromatogram see Fig. 2).

In order to compare the quality of separation and the relative duration of analysis after variations of the ionic strength and of the concentration of THF, the capacity factors (*k'*) for the standard compounds were calculated and their logarithms depicted in Fig. 3. At low salt concentrations (50 m*M*), guanine, hypoxanthine and uridine remained unresolved. The most efficient separation was obtained with 300 m*M* KH_2PO_4. Increasing amounts of THF reduced the analysis time, but worsened the resolution of uric acid, guanine and hypoxanthine. The addition of 0.1% THF turned out to yield the best separation, therefore the somewhat longer analysis time was acceptable.

Peak identification

In the plasma samples, peak identification was based on a combination of four methods.

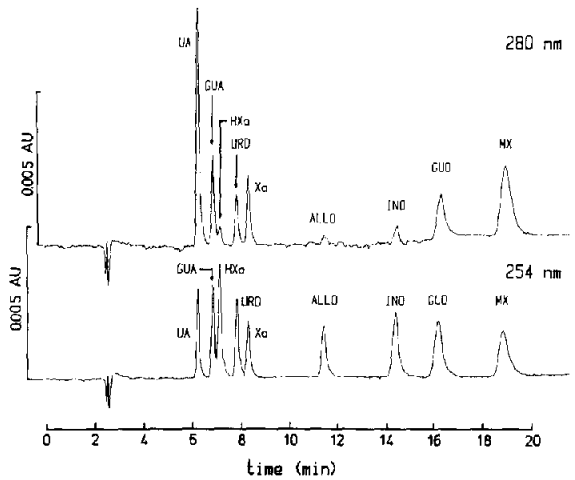

Fig. 2. Chromatograms of an aqueous standard solution recorded at 280 nm (upper panel) and at 254 nm (lower panel). Conditions: column, Nucleosil 100-5C_{18}; mobile phase, KH_2PO_4 300 m*M* (pH 4.0)–methanol–acetonitrile–THF (97.9:1:1:0.1, v/v); injection volume, 20 µl; flow-rate, 1.0 ml/min; column temperature, 30°C. Peaks: UA = uric acid; GUA = guanine; HXa = hypoxanthine, URD = uridine; Xa = xanthine; ALLO = allopurinol; INO = inosine; GUO = guanosine; MX = 7-methylxanthine; injection of 5 pmol per run (UA and MX, 10 pmol). Calibration: absorbance, 0.005 a.u.

(1) Unknown peaks were compared with standards by using absolute retention times, the retention relative to an internal standard and the capacity factors, k'. We used uric acid as an easily identifiable internal standard, because it is present in plasma in high concentrations and has a peak-height ratio greater 1. The capacity factors were calculated according to $k' = (t_R - t_0)/t_0$, where t_R is the

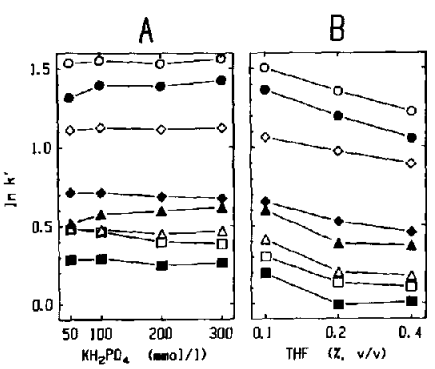

Fig. 3. Influence on capacity factors k' (ordinate ln k') of variations of ionic strength (A) and of the amount of THF in the eluent (B). Conditions: column, Nucleosil 100-5C_{18}; mobile phase A, KH_2PO_4 (pH 4.0)–methanol–acetonitrile–THF (97.9:1:1:0.1, v/v); mobile phase B, KH_2PO_4 300 m*M* (pH 4.0) methanol–acetonitrile–THF (98 − *x*:1:1:*x*, v/v); injection volume, 20 µl; flow-rate, 1.0 ml/min; column temperature, 30°C; detection wavelength, 254 nm. Symbols: (■) uric acid; (□) guanine; (△) hypoxanthine; (▲) uridine; (◆) xanthine; (◇) allopurinol; (●) inosine; (○) guanosine.

retention time of a retained and t_0 of a unretained compound, *i.e.* water, nitric acid or urea.

(2) The ratio of absorbance determined as peak height at 280 nm divided by the peak height at 254 nm is a useful characteristic for some compounds. The peak-height ratios observed in our experiments were: uric acid, 3.3; guanine, 0.9; hypoxanthine, 0.1; uridine, 0.6; xanthine, 1.2; allopurinol, 0.2; inosine, 0.3; guanosine, 0.8; 7-methylxanthine, 1.5. Although the absolute values differ, the sequence is in accordance with reported data [11,16].

(3) Enzymic peak-shift: after treatment of plasma with xanthin-oxidase (incubation at pH and temperature optimum for the enzyme as checked with standard compounds; approach according to Boulieu *et al.* [6]), the peaks thought to be hypoxanthine and xanthine disappeared from the chromatogram (data not shown).

(4) Spiking of plasma samples with standard compounds (Figs. 4C and D; [12]).

Precision of analysis

The overall precision for retention time, capacity factor, selectivity (α), peak height (h), and peak area was studied with respect to the within-day (run-to-run) and the day-to-day variation. We examined (i) aqueous standard solutions of six components with either 2 or 20 pmol per injection (20 and 200 pmol per injection for uric acid) in 44 consecutive runs in order to assess the run-to-run precision, and (ii) the 2 and 20 pmol per injection levels (20 and 200 pmol per injection for uric acid) of the calibration mixture, which was run every day together with

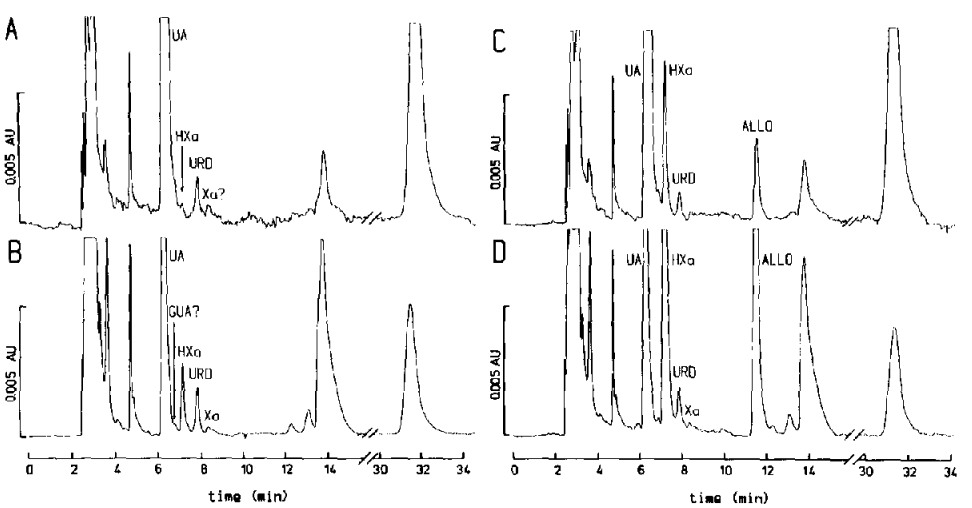

Fig. 4. Chromatograms of a patient's plasma recorded at 280 nm (A, C) and at 254 nm (B, D). In C and D, the plasma was spiked with HXa and ALLO. Conditions, peaks and calibration as in Fig. 2.

samples of human plasma, in order to assess the day-to-day precision over a period of eight days. The results of these studies are presented in Table I.

Table I shows that the repeatability of the retention time and the derived parameters k' and α in a series of 44 experiments during a single session with

TABLE I

REPEATABILITY AND REPRODUCIBILITY

Precision of the quantitation of hypoxanthine and other purines in aqueous standard. Repeatability and reproducibility of retention time (t_R), capacity factor (k'), selectivity (α), peak height (h) and peak area (at 254 nm) for uric acid (UA), guanine (GUA), hypoxanthine (HXa), uridine (URD), xanthine (Xa), allopurinol (ALLO): mean values and relative coefficient of variation (C.V., %); n indicates the number of experiments. Chromatographic conditions: Nucleosil 100-5C_{18}; mobile phase KH_2PO_4 300 mM (pH 4.0)–methanol–acetonitrile–THF (97.9:1:1:0.1, v/v); injection volume, 20 μl; flow-rate, 1.0 ml/min; column temperature 30°C (in most of the experiments). Calculations: capacity factor $k' = (t_R - t_0)/t_0$, where t_R is the retention time and t_0 the time for an unretained compound; selectivity $\alpha = k'_2/k'_1$, where $k'_2 > k'_1$.

Compound	Injected (pmol)	t_R		k'		α		h		Area	
		Mean (min)	C.V. (%)	Mean	C.V. (%)	Mean	C.V. (%)	Mean (m.a.u.)	C.V. (%)	Mean	C.V. (%)
Run-to-run precision (n=44)											
UA	20	6.64	0.05	1.43	0.08			6.66	0.9	0.97	0.9
GUA	2	7.40	0.04	1.70	0.07	1.19	0.02	1.43	1.3	0.22	2.8
HXa	2	7.66	0.04	1.80	0.07	1.06	0.02	1.71	0.8	0.30	1.9
URD	2	8.03	0.04	1.94	0.07	1.08	0.01	1.32	1.1	0.23	2.5
Xa	2	8.83	0.05	2.23	0.08	1.15	0.02	0.81	1.4	0.15	3.1
ALLO	2	12.04	0.06	3.40	0.08	1.53	0.02	0.80	1.3	0.15	3.5
UA	200	6.66	0.10	1.44	0.17			65.26	0.6	8.87	3.6
GUA	20	7.43	0.06	1.72	0.10	1.19	0.20	14.10	0.3	2.04	2.5
HXa	20	7.69	0.06	1.82	0.09	1.06	0.10	16.96	0.3	2.92	0.4
URD	20	8.06	0.06	1.95	0.09	1.07	0.01	12.79	0.4	2.38	0.5
Xa	20	8.87	0.07	2.25	0.10	1.15	0.02	7.58	0.4	1.36	0.7
ALLO	20	12.08	0.08	3.43	0.11	1.52	0.11	7.67	0.5	1.67	4.4
Day-to-day precision (n=8)											
UA	20	6.67	1.93	1.44	2.59			6.49	5.8	1.00	2.9
GUA	2	7.36	1.38	1.70	1.71	1.18	1.27	1.34	5.9	0.21	3.6
HXa	2	7.63	1.44	1.80	1.76	1.06	0.57	1.57	7.2	0.28	5.6
URD	2	8.12	1.61	1.98	2.03	1.10	0.58	1.16	6.4	0.21	5.0
Xa	2	8.84	1.62	2.24	1.90	1.13	0.54	0.61	7.6	0.10	8.7
ALLO	2	12.09	1.90	3.43	2.07	1.53	0.20	0.74	5.9	0.14	5.3
UA	200	6.71	2.15	1.45	3.35			65.90	6.1	9.93	1.3
GUA	20	7.39	1.50	1.71	2.03	1.18	1.36	12.35	5.7	1.94	3.3
HXa	20	7.68	1.58	1.81	2.17	1.06	0.54	14.84	6.6	2.72	3.9
URD	20	8.16	1.75	1.99	2.44	1.10	0.55	10.73	6.5	2.12	4.8
Xa	20	8.89	1.78	2.25	2.34	1.13	0.51	5.60	8.7	1.08	7.6
ALLO	20	12.18	2.12	3.46	2.54	1.52	0.38	6.36	6.0	1.62	5.1

identical reagents and equipment is excellent (C.V. < 0.2%). The run-to-run precision of the peak height (C.V. 0.4–1.4%) is considerably better than that of the peak area (C.V. 0.4–4.4%). This is probably caused by integration errors due to difficulties in defining the start and end of a peak, because of baseline noise. Nevertheless, the repeatability of the retention and peak-height data meets the quality criteria established by other authors [27,28].

The results of identical experiments made on eight different days were less precise, *i.e.* the reproducibility of retention data (t_R, k', α) showed errors in the range 1–3%. With respect to peak height and peak area, the reproducibility was even worse (C.V. 2–8%). The overall variability reported by other authors is lower by a factor of *ca.* 2 [12,18,21,22,27,28]. The reasons for these large variances on different days are (i) variability in the calibration because a fresh serial dilution of stock solution had to be prepared each day, (ii) variability in the preparation of mobile phases with identical composition and (iii) temperature fluctuations. Of these factors, the temperature control has the greatest impact on the reproducibility, because thermal changes influence the flow-rate, retention parameters, mobile phase composition, baseline noise, etc. [27,28]. Although the column temperature was kept constant at 30.0 ± 0.5°C, we had to perform our experiments in a laboratory without air-conditioning during the summer months, *i.e.* we had to face temperature fluctuations of ±4°C, which could easily account for the large variations in reproducibility.

In the day-to-day experiments, the mean amplitude of the baseline noise was $1.72 \cdot 10^{-4}$ a.u. (C.V. 8.5%). Assuming a signal-to-noise ratio of 2, and calculating the linearity of the concentration–response relation (correlation coefficients, $r > 0.99$ for all purine derivatives as calculated by least-squares regression analysis), the following mean detection limits could be calculated (in pmol per injection): uric acid, 1.14 (C.V. 6.8%); guanine, 0.54 (C.V. 4.9%); hypoxanthine, 0.46 (C.V. 5.4%); uridine, 0.63 (C.V. 5.8%); xanthine, 1.22 (C.V. 7.5%), allopurinol, 1.02 (C.V. 5.6%). The detection limits for hypoxanthine and xanthine reported in the literature are of the same order of magnitude [6,18,21,22]. Our results were obtained with a single Nucleosil column, which had a lifetime of more than 600 injections (for plasma *ca.* 400). Periodic regeneration of the column with methanol–water (70:30, v/v) was sufficient to eliminate ghost peaks and drifting baselines, and to maintain the performance of the column in the range 50 000–65 000 theoretical plates per metre (63 000 as stated by the manufacturer).

Efficiency of extraction

The efficiency of extraction of purine-related compounds, especially of hypoxanthine, was studied by determination of the recovery of tritiated hypoxanthine added to samples of human pool plasma. Two methods of deproteinization were compared, *i.e.* protein precipitation with TCA–Freon and protein removal by ultrafiltration. In our hands, the TCA–Freon method yielded a recovery of 86 ± 0.86% (mean ± S.D., $n = 10$) of [^{3}H]hypoxanthine in the final supernatant,

which is comparable with the 92% of [^{14}C]hypoxanthine recovered after perchloric acid extraction [2]. The remaining 14% of the [^{3}H]hypoxanthine is found in the fractions of the protein precipitate (10%) and the trioctylamine–Freon (4%). However, it should be noted that the [^{3}H]hypoxanthine found in the precipitate and the Freon is most likely due to aqueous contamination of these phases, since it is very difficult to remove the supernatants completely. The loss of [^{3}H]hypoxanthine could be reduced to negligible amounts if the plasma proteins were removed by ultrafiltration (recovery 96 ± 0.96%, $n = 4$). Therefore, our extraction method is as efficient as those in other reports [2,9,13,17,29] or even better [30].

Application to plasma

As can be seen from the typical chromatograms in Fig. 4, human plasma contains a lot of low-molecular-mass substances that elute earlier than uric acid. We made no attempt to identify these. The peaks eluting just after uric acid, *i.e.* guanine, hypoxanthine, uridine and xanthine, were of greater interest; they are all baseline-separated from each other, with the exception of guanine, which forms a small shoulder on the back of the uric acid peak and therefore was neglected. In contrast to the aqueous standard, one run was completed within 35 min, since one unidentified peak constantly appeared after *ca.* 32 min. Although the chromatogram contained a multitude of unidentified peaks, we did not feel the necessity to identify hypoxanthine and other substances positively in every sample by enzymic peak shift, as stated by Ontyd and Schrader [14], since the pattern of the chromatogram was very consistent between the individuals studied.

The quantitation of hypoxanthine in human arterial and venous plasma samples revealed that under control conditions, *i.e.* before cardioplegia and before aortic cross-clamping, the hypoxanthine levels are low and not largely different in arterial and venous samples (arterial, 1.4 ± 0.3 μM; venous, 3.4 ± 1.0 μM; mean ± S.D., $n = 8$). These observations are consistent with previous results (for a review see ref. 1). The hypoxanthine levels measured in coronary venous blood are significantly increased in samples withdrawn 10 min after removal of the aortic cross-clamp (18.8 ± 7.0 μM, mean ± S.D., $n = 8$, $p < 0.05$) and decline again to control levels over several hours. These changes are less prominent in arterial samples, where the hypoxanthine levels nearly double before reaching basal levels again. In comparison, the hypoxanthine levels measured in peripheral venous blood of healthy volunteers were lower than in diseased subjects and very close to the detection limit (0.5 ± 0.1 μM, mean ± S.D., $n = 9$). The increased hypoxanthine levels can be interpreted as indicative of perioperative ischemic periods [4,5,31] or the degradation of energy-rich phosphates in critically ill patients [15].

ACKNOWLEDGEMENTS

We thank K. Belsner (Janssen Research Foundation, Neuss, Germany) for helpful discussions, and Doris Petermeyer for her invaluable technical assistance.

REFERENCES

1 R. A. Harkness, *J. Chromatogr.*, 429 (1988) 255.
2 E. E. F. Harmsen, P. P. de Tombe, J. W. de Jong and P. W. Achterberg, *Am. J. Physiol.*, 246 (1984) H37.
3 E. A. Hull-Ryde, W. R. Lewis, C. D. Veronee and J. E. Lowe, *J. Chromatogr.*, 377 (1986) 165.
4 W. J. Remme, R. van den Berg, M. Mantel, P. H. Cox, D. C. A. van Hoogenhuyze, X. H. Krauss, C. J. Storm and D. A. C. M. Kruyssen, *Am. J. Cardiol.*, 58 (1986) 1188.
5 P. W. Serruys, H. Suryapranata, F. Piscione, E. Harmsen, M. van den Brand, P. de Feyter, P. G. Hugenholtz, and J. W. de Jong, *Am. J. Cardiol.*, 63 (1989) 45E.
6 R. Boulieu, C. Bory, P. Baltassat and C. Gonnet, *J. Chromatogr.*, 233 (1982) 131.
7 M. E. Dwyer and P. R. Brown, *J. Chromatogr.*, 345 (1985) 125.
8 E. Harmsen, J. W. de Jong and P. W. Serruys, *Clin. Chim. Acta*, 115 (1981) 73.
9 M. Kito, R. Tawa, S. Takeshima and S. Hirose, *J. Chromatogr.*, 278 (1983) 35.
10 M. Zakaria and P. R. Brown, *Anal. Biochem.*, 120 (1982) 25.
11 R. A. Hartwick, S. P. Assenza and P. R. Brown, *J. Chromatogr.*, 186 (1979) 647.
12 R. A. Hartwick, A. M. Krstulovic and P. R. Brown, *J. Chromatogr.*, 186 (1979) 659.
13 A. McBurney and T. Gibson, *Clin. Chim. Acta*, 102 (1980) 19.
14 J. Ontyd and J. Schrader, *J. Chromatogr.*, 307 (1984) 404.
15 C. M. Grum, R. H. Simon, D. R. Dantzker and I. H. Fox, *Chest*, 88 (1985) 763.
16 C. W. Gehrke, K. C. Kuo and R. W. Zumwalt, *J. Chromatogr.*, 188 (1980) 129.
17 H. Miyazaki, Y. Matsunaga, K. Yoshida, S. Arakawa and M. Hashimoto, *J. Chromatogr.*, 274 (1983) 75.
18 F. Niklasson, *Clin. Chem.*, 29 (1983) 1543.
19 W. E. Wung and S. B. Howell, *Clin. Chem.*, 26 (1980) 1704.
20 R. Boulieu, C. Bory, P. Baltassat and C. Gonnet, *Anal. Biochem.*, 129 (1983) 398.
21 R. Boulieu, C. Bory, P. Baltassat and C. Gonnet, *J. Chromatogr.*, 307 (1984) 469.
22 R. J. Simmonds and R. A. Harkness, *J. Chromatogr.*, 226 (1981) 369.
23 J. Wynants, B. Petrov, J. Nijhof and H. van Belle, *J. Chromatogr.*, 386 (1987) 297.
24 L. Sachs, *Angewandte Statistik*, Springer, Berlin, 1984.
25 A. P. Halfpenny and P. R. Brown, in H. Engelhardt (Editor), *Practice of High Performance Liquid Chromatography. Applications, Equipment, and Quantitative Analysis*, Springer, Heidelberg, 1986, p. 323.
26 R. J. Henderson and C. A. Griffin, *J. Chromatogr.*, 226 (1981) 202.
27 R. P. W. Scott and C. E. Reese, *J. Chromatogr.*, 138 (1977) 283.
28 E. Grushka and I. Zamir, in P. R. Brown and R. A. Hartwick (Editors), *High Performance Liquid Chromatography*, Wiley, New York, 1989, p. 529.
29 J. Blanchard, *J. Chromatogr.*, 226 (1981) 455.
30 R. A. Hartwick, D. van Haverbeke, M. McKeag and P. R. Brown, *J. Liq. Chromatogr.*, 2 (1979) 725.
31 A. Edlund, B. Berglund, D. van Dorne, L. Kaijser, J. Nowak, C. Patrono, A. Sollevi and A. Wennmalm, *Circulation*, 71 (1985) 1113.