Journal of Chromatography, 425 (1988) 121–134
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4000

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF α-AMANITIN AND PHALLOIDIN IN HUMAN PLASMA USING THE COLUMN-SWITCHING TECHNIQUE AND ITS APPLICATION IN SUSPECTED CASES OF POISONING BY THE GREEN SPECIES OF AMANITA MUSHROOM (*AMANITA PHALLOIDES*)

W. RIECK* and D. PLATT

Institut für Gerontologie der Universität Erlangen-Nürnberg und 2. Medizinische Klinik, Klinikum Nürnberg, Nürnberg (F.R.G.)

(First received May 19th, 1987; revised manuscript received October 8th, 1987)

SUMMARY

A reversed-phase high-performance liquid chromatographic assay has been developed for the simultaneous determination of α-amanitin and phalloidin in human plasma. The procedure is based on the enrichment of the toxins on a pre-column, followed by the transfer of both compounds in a foreflush mode to the analytical column. α-Amanitin and phalloidin can be quantified reliably down to a minimum concentration of 10 ng/ml in plasma (relative standard deviation < 10%). An alternative method is recommended for hepatic coma patients.

INTRODUCTION

Even today the diagnosis of suspected poisoning by the mushroom *Amanita phalloides* is still a challenge to the clinician [1,2]. Among the constituents of the mushroom the amatoxins have proved to be a class of substances that are deadly poisonous to humans. Also poisonous, but to a lesser extent, are the phallotoxins, of which phalloidin is the biochemically most active representative [3–5].

The radioimmunoassay (RIA) methods developed by Faulstich and co-workers [6,7] and Fiume et al. [8] for the determination of α-amanitin in urine require a relatively long period (determined by the 2-h incubation time) before analytical data are available*.

*After having completed the work presented in this paper, a communication by Andres et al. [9] appeared in which a new RIA method was presented that reputedly circumvents the difficulties encountered in previously established RIA assays.

0378-4347/88/$03.50

An alternative method for the determination of the toxic principles of poisonous mushrooms (α-amanitin in the amatoxin group of substance and phalloidin in the phallotoxin group of substances) is provided by high-performance liquid chromatography (HPLC). A first step in this direction was undertaken by Pastorello et al. [10]. The HPLC methods published since that time, however, are either too tedious in the sample preparation stage [11] or they still do not offer any advantage over the conventional RIA methods regarding detection sensitivity [12]. In addition, in biological samples of clinical relevance we sometimes observed endogenous interferences in the lower nanogram concentration range of α-amanitin. To our knowledge, no reliable identification and quantification by means of simple HPLC gradient elution methods has been published [13].

Our requirements for a detection method, namely rapidity, a sensitivity comparable to that of ^{3}H RIA methods [6–8], selectivity and the simultaneous determination of α-amanitin and phalloidin proved to be a difficult problem.

The problem could be solved by three decisive factors:

(1) α-Amanitin and phalloidin could be enriched by relatively large aqueous injection volumes (up to 2 ml) on an RP-8 Spheri-5 pre-column (3 cm $\times$ 2.1 mm I.D.).

(2) The column-switching technique which, in principle, suffices with only one automatically switchable valve, could be used for the determination of α-amanitin and phalloidin.

(3) There is the possibility of cleaning and equilibrating the pre-column during the analysis, so that a renewed injection can be carried out after ca. 35 min.

EXPERIMENTAL

Reagents and solvents

α-Amanitin (AMA) and silibinin were supplied by Dr. Madaus & Co. (Cologne, F.R.G.). Phalloidin (PHA) was obtained from Sigma (Munich, F.R.G.) and 5-sulphosalicylic acid dihydrate, acetic acid and sodium acetate from Merck (Darmstadt, F.R.G.), all of analytical-reagent grade. Water and acetonitrile (both of HPLC grade) were obtained from Promochem (Wesel, F.R.G.).

An α-amanitin stock solution was prepared by dissolution of 10 mg of α-amanitin in 10 ml of water and a phalloidin stock solution by dissolution of 1 mg of phalloidin in 10 ml of water. Standard solutions AMA-1 (10 μg/ml) and AMA-2 (5 μg/ml) were prepared from the α-amanitin stock solution by dilution of 100 and 50 μl, respectively, with water to a final volume of 10 ml in each instance. Dilution of 2 and 1 ml, respectively, of the standard solution AMA-1 to 10 ml in each instance provided the additional standard solutions AMA-3 (2 μg/ml) and AMA-4 (1 μg/ml), respectively. Dilution of 1 ml, 500 μl, 200 μl and 100 μl, respectively, of the phalloidin stock solution with water to 10 ml in each instance furnished the phalloidin standard solutions PHA-1 (10 μg/ml), PHA-2 (5 μg/ml), PHA-3 (2 μg/ml) and PHA-4 (1 μg/ml).

The following solutions were required for work-up of the plasma samples:

Solution A. A 5.08-g (20 mmol) amount of 5-sulphosalicylic acid dihydrate was

dissolved in 5 ml of water and the thoroughly mixed solution was stored in the dark at room temperature. A fresh solution was prepared each week.

Solution B. A 16.4-g (0.2 mol) amount of sodium acetate was dissolved in 12.0 g (0.2 mol) of acetic acid and the solution was diluted with water to 100 ml.

Instrumental

A schematic representation of the column-switching set-up is given in Fig. 1.

The eluent A (1 ml of acetonitrile plus 1 l of water) was delivered by a single-piston pump PA (Model 410; Kontron, Eching, F.R.G.) using a flow programme

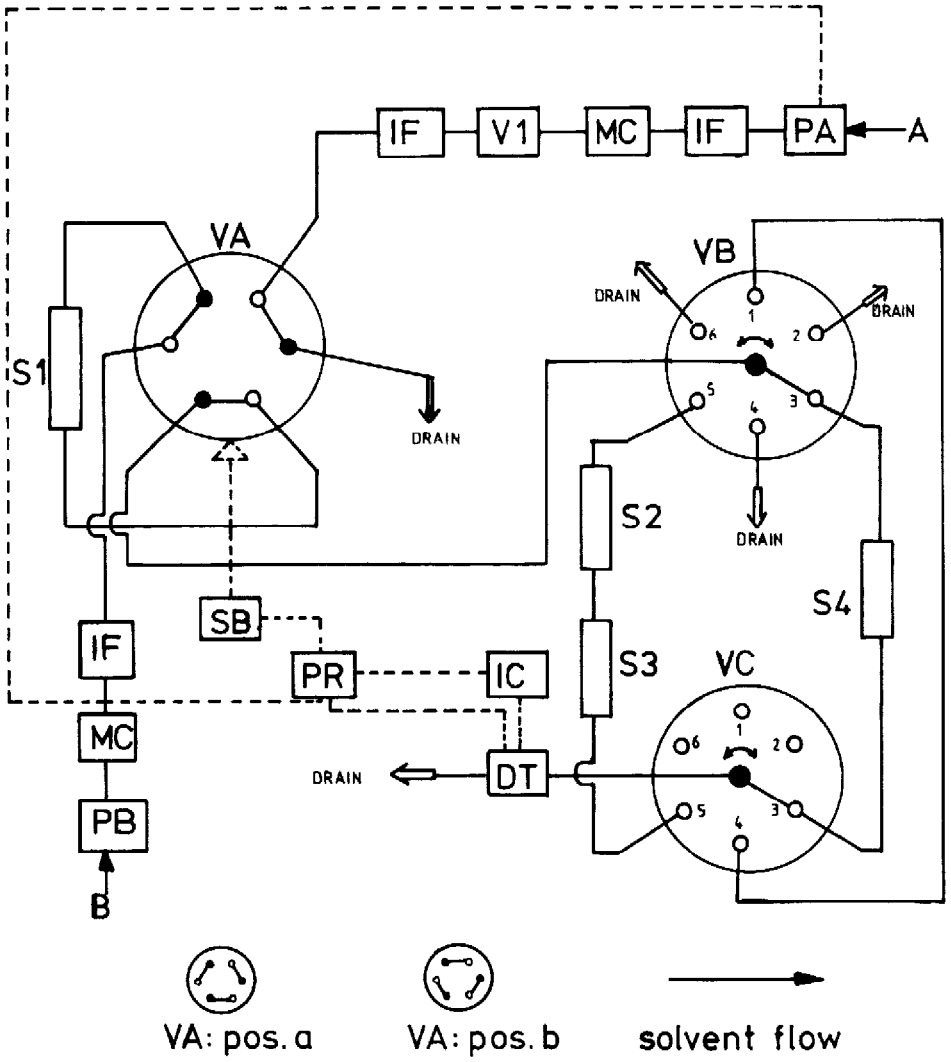

Fig. 1. Schematic representation of the column-switching system for the analysis of α-amanitin and phalloidin. Stainless-steel capillary tubing (0.12 mm I.D.) was used for connecting the parts following the sample injector V1 and the inlet filter IF in the line of pump PB. For further details, see text.

and was passed through a column inlet filter IF (Type 7302, 2-μm frit; Rheodyne, Cotati, CA, U.S.A.) via a mixing chamber MC (Kontron) into the injection valve V1 (Type 7125, fitted with a 2-ml sample loop; Rheodyne), with which 2 ml of

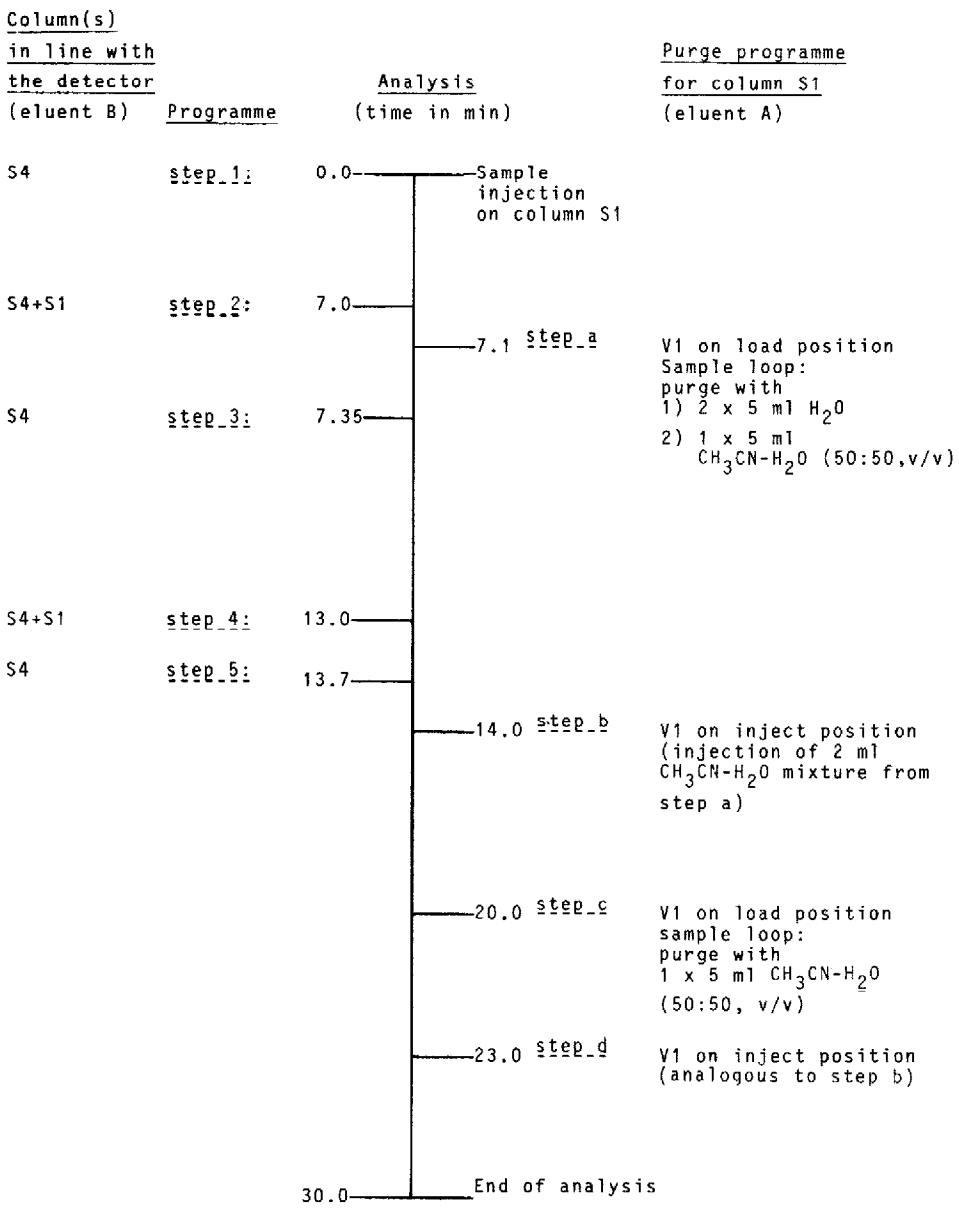

Fig. 2. Schematic representation of programme A: VB = 3; VC = 3; PB = 1.0 ml/min. Step 1: 0.0–7.0 min; VA = b; PA = 0.5 ml/min. Step 2: 7.0–7.35 min; VA = a; PA at 7.1 min, 0.3 ml/min; PA at 7.3 min, flow 0. Step 3: 7.35–13.0 min; VA = b; PA, flow 0. Step 4: 13.0–13.7 min; VA = a; PA at 13.1 min, 0.3 ml/min; PA at 13.5 min, 0.5 ml/min. Step 5: 13.7–30.0 min; VA = b; PA = 0.5 ml/min. For the purge programme a 5.0-ml gas-tight Hamilton syringe (Model 1005 LTN) was used.

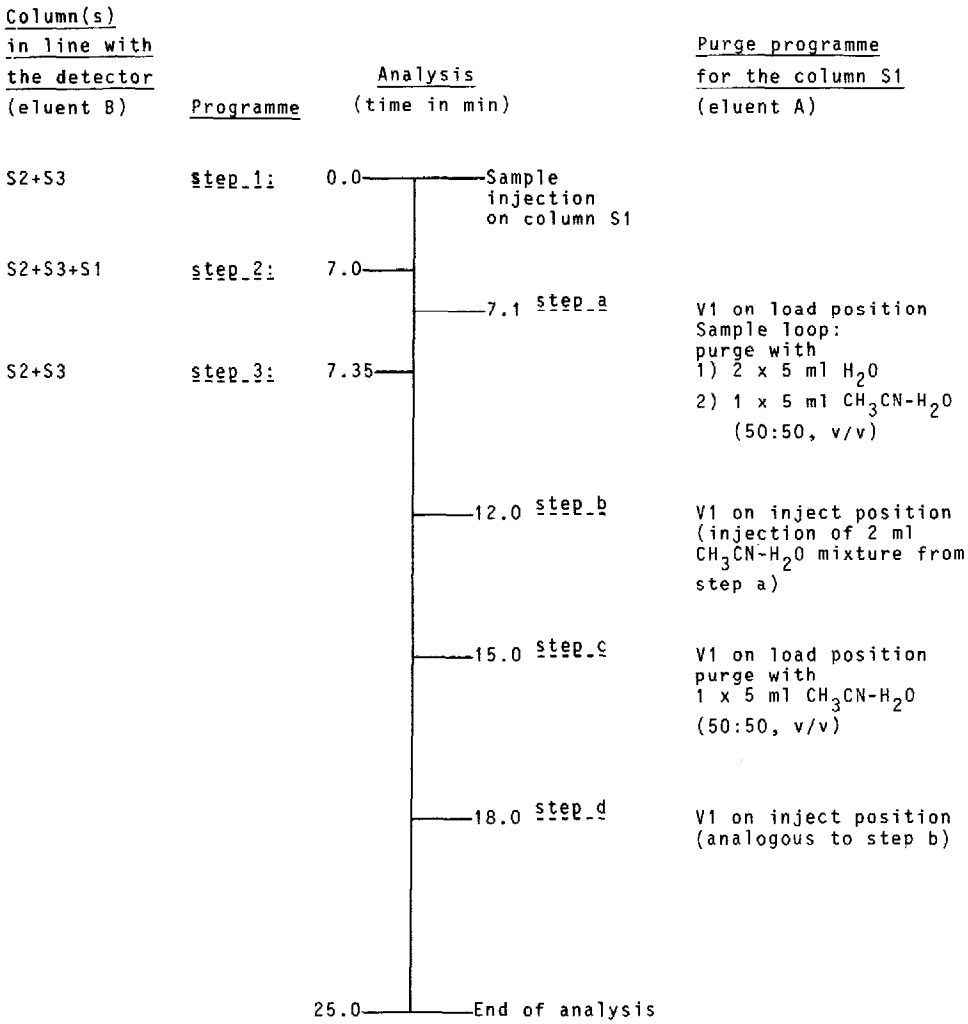

Fig. 3. Schematic representation of programme B: VB = 5; VC = 5; PA = 0.5 ml/min; PB = 1.0 ml/min. Step 1: 0–7.0 min; VA = b. Step 2: 7.0–7.35 min; VA = a. Step 3: 7.35–25.0 min; VA = b.

the sample solution were injected into the chromatographic system. The eluent A then transported the injected sample via a further column inlet filter IF on to the pre-column S1 (MPLC cartridge RP-8 Spheri-5, particle size 5 μm, 3 cm $\times$ 2.1 mm I.D.; Kontron), which was fitted by means of a Brownlee cartridge holder system to the automatic valve VA (Type 7000, fitted with a pneumatic actuator and a 12-V D.C. solenoid valve; Rheodyne). Enrichment of the α-amanitin and phalloidin from the injected sample solution was achieved on column S1.

Eluent B [2000 ml of water plus 400 ml of acetonitrile, i.e., acetonitrile–water (16.67 : 83.33, v/v)] was delivered by pump PB (Model 414T; Kontron) at a flow-rate of 1 ml/min via a mixing chamber MC and a column-inlet filter IF and then transported the enriched α-amanitin and phalloidin from column S1 to the analytical column S4 (MPLC cartridge RP-8 Spheri-5, particle size 5 μm, 10 cm $\times$ 4.6

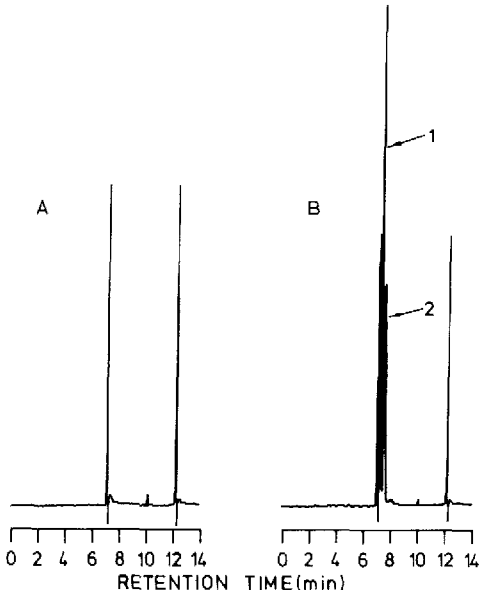

Fig. 4. Chromatograms of (A) water and (B) test sample QC (100 ng/ml each of α-amanitin and phalloidin), analysed by programme D. Peaks: 1 = α-amanitin, retention time 7.30 min; 2 = phalloidin, retention time 7.56 min. Chromatographic conditions (cf. Fig. 1): pre-column S1, RP-8 Spheri-5, particle size 5 μm, 3 cm × 2.1 mm I.D.; injection volume, 2 ml; detection, 303 nm, 0.064 a.u.f.s.; eluent A, acetonitrile–water (1:1000, v/v); eluent B, acetonitrile–water (16.67:83.33, v/v); chart speed, 0.5 cm/min. Programme D: VB = 1; VC = 4; PA = 0.5 ml/min; PB = 1.0 ml/min. Step 1: 0–7.0 min; VA = b. Step 2: 7.0–10.0 min; VA = a. Step 3: 10.0–12.0 min; VA = b. Step 4: 12.0–20.0 min; VA = a.

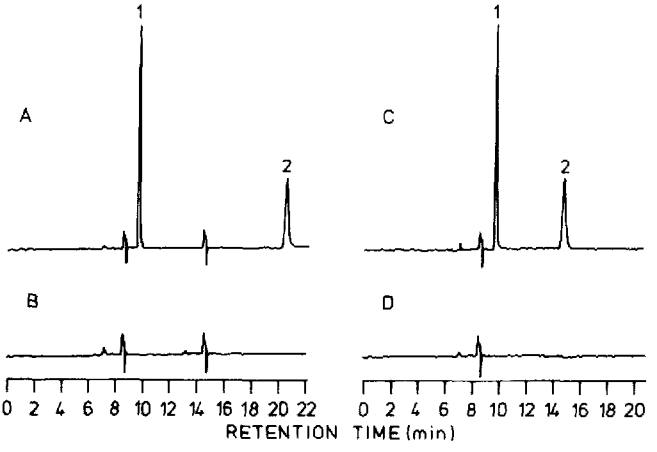

Fig. 5. Chromatograms of (A) test sample QC (analysed by programme A without purge steps), (B) water (programme A without purge steps), (C) test sample QC (programme C) and (D) water (programme C). Peaks: 1 = α-amanitin, retention time 9.72 min; 2 = phalloidin, retention time (A) 20.5 min and (C) 14.76 min. Chromatographic conditions as in Fig. 4, except analytical column S4, RP-8 Spheri-5, particle size 5 μm, 10 cm × 4.6 mm I.D. Programme C: VB = 3; VC = 3; PA = 0.5 ml/min; PB = 1.0 ml/min. Step 1: 0–7.0 min; VA = b. Step 2: 7.0–7.7 min; VA = a. Step 3: 7.7–20.0 min; VA = b.

mm I.D.; Kontron). Alternatively, the α-amanitin was transferred to the column combination S2 (MPLC cartridge RP-8 Spheri-5, particle size 5 μm, 10 cm × 4.6 mm I.D.; Kontron) and S3 (MPLC cartridge RP-18 Spheri-5, particle size 5 μm, 10 cm × 4.6 mm I.D.; Kontron). The columns S2, S3 and S4 were likewise fitted by means of a Brownlee cartridge holder system to the valves VB and VC, respectively (both Type 7060 valves, manually switchable; Rheodyne), with unions [Type ZU1C (Valco), obtained from Kontron] installed between the column exits of S3 and S4 and the entry to the valve VC.

Detection of the α-amanitin and phalloidin was carried out with a UV detector DT (Model Uvikon 720 LC, 2.8-μl cell; Kontron) at a wavelength of 303 nm. An integrator IC (Model SP 4100; Spectra-Physics, Darmstadt, F.R.G.) was used for recording and evaluating the resulting chromatograms.

The switchbox SB (Model S 112; ERC, Alteglofsheim, F.R.G.) had to be installed between the valve VA and the programmer PR so that the valve VA could be switched by the programmer PR (Model 200; Kontron).

Analytical procedure

The analysis (α-amanitin and phalloidin analysed on columns S1 and S4; programme A) required 30 min and involved the steps outlined schematically in Fig. 2.

For additional confirmation of the analytical results and in problematic cases our method offers the possibility of alternatively analysing the α-amanitin with the column combination S2 and S3 within 25 min (programme B). A schematic diagram of the analysis by this method for the determination of α-amanitin is given in Fig. 3.

The chromatographic efficiency of columns S1 and S4 and the reproducibility of the column-switching processes at S1 were checked using both programme A [without purging programme, test sample (quality control, QC) injected] and programmes C and D (see Figs. 4 and 5).

Preparation of plasma calibration samples

Venous blood (containing neither α-amanitin nor phalloidin) was centrifuged for 10 min at 3000 *g* in a heparinized test-tube. Samples of 3 ml of the separated plasma were treated in 10-ml ground-glass test-tubes or in serum tubes (Sarstedt, Nümbrecht, F.R.G.), first with of the standard solutions, 30 μl each of AMA-1 and PHA-1 or AMA-2 and PHA-2, AMA-3 and PHA-3, and AMA-4 and PHA-4, then diluted with 1 ml of water and mixed for 1 min. The concentrations of α-amanitin and phalloidin, calculated per 3 ml of plasma, were 100, 50, 20 and 10 ng/ml, respectively. Each of the spiked plasma samples was finally treated with 150 μl of the sulphosalicylic acid solution (solution A), stirred for 1 min, allowed to stand for 5 min at room temperature and then centrifuged for 15 min at 3000 *g*. A 3-ml volume of the resulting supernatant liquor was divided equally between three Eppendorf reaction vessels and each portion was treated with 100 μl of acetate buffer (solution B) and mixed for 1 min. After subsequent centrifugation (2 min at 10 000 *g*, Eppendorf centrifuge, Model 3200) the supernatants were

combined in a brown 4-ml vial and the sample was injected via valve V1 into the chromatographic system.

For the determination of the recovery, 3-ml samples of water were treated, as described above for the plasma samples, with α-amanitin and phalloidin and then with 1 ml of water. After thorough mixing for 1 min the samples were injected into the chromatographic system, but this time directly without further workup.

Preparation of patients' plasma samples

Patients' plasma was isolated as described for the plasma calibration samples. A 3-ml volume of the plasma was then treated with 1.06 ml of water and the mixture was likewise thoroughly mixed for 1 min prior to further workup as for the plasma calibration samples.

Quality control (QC) samples

Aliquots of 300 μl of the standard solutions AMA-1 and PHA-1 were transferred into 30-ml measuring flasks and diluted to the 30-ml mark with water. The solutions, if not required for injection on the same day, were transferred into brown 4-ml vials and stored at 4°C. The QC solutions contained 100 ng/ml each of α-amanitin and phalloidin.

Injection of samples

The following procedure has proved satisfactory for ensuring reproducible injection of the samples into the chromatographic system via valve V1:

(1) The 2-ml sample loop was filled with water using a 2.5-ml gas-tight syringe (Model 1002 LTN; Hamilton Deutschland, Darmstadt, F.R.G.).

(2) A 2.5-ml volume of the sample solution was then injected through the sample loop at a constant rate of ca. 50 μl/s. After a further 10 s, the valve V1 was switched from the load to the injection position.

RESULTS AND DISCUSSION

Chromatographic efficiency of the pre-column S1 and precision of the switching processes checked with the test sample (QC)

For incorporation of the column switching process in the chromatographic analysis, it was essential to demonstrate the constancy of the retention times of α-amanitin and phalloidin on the pre-column S1. If one injects the test sample QC into the chromatographic system and uses the programme D, chromatograms of the type shown in Fig. 4B are obtained. The relevant experimental data are given in Table I.

Differences in retention times were just evident with phalloidin and to a much lesser extent with α-amanitin. Thus the column with charge No. 04304 even showed a decrease in the retention time (t_R) for phalloidin from 7.53 [within-day determination of phalloidin; $t_R = 7.53 \pm 0.005$ min; relative standard deviation (R.S.D.) = 0.13%, $n = 4$] to 7.42 min, associated with an increase in the back-pressure from initially ca. 50 bar to about 80 bar.

This retention behaviour of phalloidin was the reason why the eluent B re-

TABLE I

PRECISION OF RETENTION TIMES OF α-AMANITIN AND PHALLOIDIN TEST SAMPLES QC ANALYSED ON PRE-COLUMN S1 LOADED WITH PLASMA SAMPLES, USING PROGRAMME D

Alternating injections on pre-column S1: charge No. 10185A, 38 plasma samples, 153 samples in total; charge No. 04304, (a) 18 plasma samples, 101 samples in total; (b) 33 plasma samples, 117 samples in total.

Compound	Charge No.	Retention time (min)		R.S.D. (%)	*n*	Test period (days)
		Mean ± S.E.M.	S.D.			
α-Amanitin	10185A	7.32 ± 0.003	0.014	0.19	30	10
	04304	7.23 ± 0.005	0.021	0.30	22	7
	04304	7.25 ± 0.008	0.029	0.40	15	15
Phalloidin	10185A	7.57 ± 0.005	0.028	0.37	30	10
	04304	7.42 ± 0.017	0.082	1.10	22	7
	04304	7.57 ± 0.018	0.070	0.92	15	15

maining in the inlet capillary to column S1 after the first switching process (programme A) suffices to substantially lower the detector response for phalloidin during the test interval. This difficulty could be overcome by incorporation of a "stop flow" step in programme A (step 2).

In spite of the individual differences in the retention times of α-amanitin and phalloidin in the tested pre-columns S1, the switching times used in programme A were chosen to be adequately large to obtain a very good reproducibility for the α-amanitin and phalloidin peak areas. The correctly chosen switching times in programme A could also be checked, by transferring α-amanitin and phalloidin together and not separately from the pre-column S1 to the analytical column S4 (programme C, test sample QC).

Typical chromatograms obtained for test sample QC according to programme A (without a purging programme) and programme C are shown in Fig. 5A and C, respectively. In addition, the experimental results show that it is possible to analyse five plasma samples one after another (programme A or B) on a single pre-column. Then the pre-column S1 has to be equilibrated with an additional 4 ml of acetonitrile–water (50:50, v/v; programme D). In this way, a total of about 30, but no more than 40, plasma samples per pre-column can be analysed.

Plasma sample preparation

The reason for the limited number of plasma sample injections via the pre-column S1 was the very high sulphosalicylic acid concentration. On the other hand, this sample preparation was necessary in order to meet the aforementioned time factor required for the total analysis time. If the sulphosalicylic acid concentration is lowered then the pre-column S1 will of course be less loaded, but the analytical solutions exhibited erratic retarded precipitation.

Calibration graph

The calibration graph determined with the aid of programme A was linear in the concentration range 10–100 ng/ml for both α-amanitin and phalloidin (linear regression analysis). By means of a correlation calculation, correlation coefficients (r) greater than 0.999 were obtained for both α-amanitin and phalloidin.

Precision and recovery

The efficiency of the method is shown in Table II, in which precision and recovery data are given.

Both the recovery, which represents a measure of the accuracy at a given selectivity, and the good precision clearly demonstrate that one can use the column-switching technique for the determination of α-amanitin and phalloidin. The recovery of α-amanitin and phalloidin is ca. 70% in each instance in the concen-

TABLE II

PRECISION AND RECOVERY FOR PLASMA SAMPLES ANALYSED USING PROGRAMME A

Compound	Actual concentration (ng/ml)	Measured concentration (ng/ml)		R.S.D. (%)	Recovery (%)
		Mean ± S.E.M.	S.D.		
Within-day ($n=3$)					
α-Amanitin	50.0	49.37 ± 1.33	2.31	4.68	98.74
	100.0	100.04 ± 0.72	1.24	1.24	100.04
Phalloidin	50.0	50.34 ± 0.71	1.24	2.45	100.68
	100.0	99.23 ± 0.27	0.46	0.47	99.23
Between-day ($n=6$)					
α-Amanitin	50.0	52.78 ± 1.78	4.36	8.26	105.56
	100.0	96.14 ± 2.31	5.65	5.87	96.14
Phalloidin	50.0	48.18 ± 1.28	3.14	6.51	96.36
	100.0	94.53 ± 2.19	5.37	5.68	96.53

TABLE III

RECOVERY AND PRECISION OF RETENTION TIMES FOR PLASMA SAMPLES ANALYSED USING PROGRAMME A

Compound	Concentration (ng/ml)	Recovery (mean ± S.D., $n=6$) (%)	Retention time			
			Within-day ($n=3$)		Between-day ($n=6$)	
			Mean ± S.D. (min)	R.S.D. (%)	Mean ± S.D. (min)	R.S.D. (%)
α-Amanitin	50.0	67.3 ± 5.4	9.71 ± 0.01	0.12	9.71 ± 0.01	0.09
	100.0	68.7 ± 3.9	9.71 ± 0.01	0.10	9.71 ± 0.01	0.11
Phalloidin	50.0	74.9 ± 1.8	20.44 ± 0.06	0.27	20.43 ± 0.05	0.25
	100.0	74.0 ± 5.2	20.43 ± 0.06	0.30	20.40 ± 0.06	0.30

tration range 10–100 ng/ml. The data determined for concentrations of 50 and 100 ng/ml are listed in Table III. Also given in Table III are the experimental results obtained for the precision of the retention times of α-amanitin and phalloidin in plasma samples on the analytical column S4. In connection with the good peak shape (see Fig. 5), the constancy of the retention time represents a criterion for the identification of α-amanitin. As an identity criterion the permitted retention time range included values up to ten times of the standard deviation of the retention times (9.66–9.76 min; see Table III).

Limit of detection and selectivity

Both α-amanitin and phalloidin can be reliably determined down to a minimum concentration of 10 ng/ml in plasma (α-amanitin, $\bar{x}$=9.74 ng/ml,

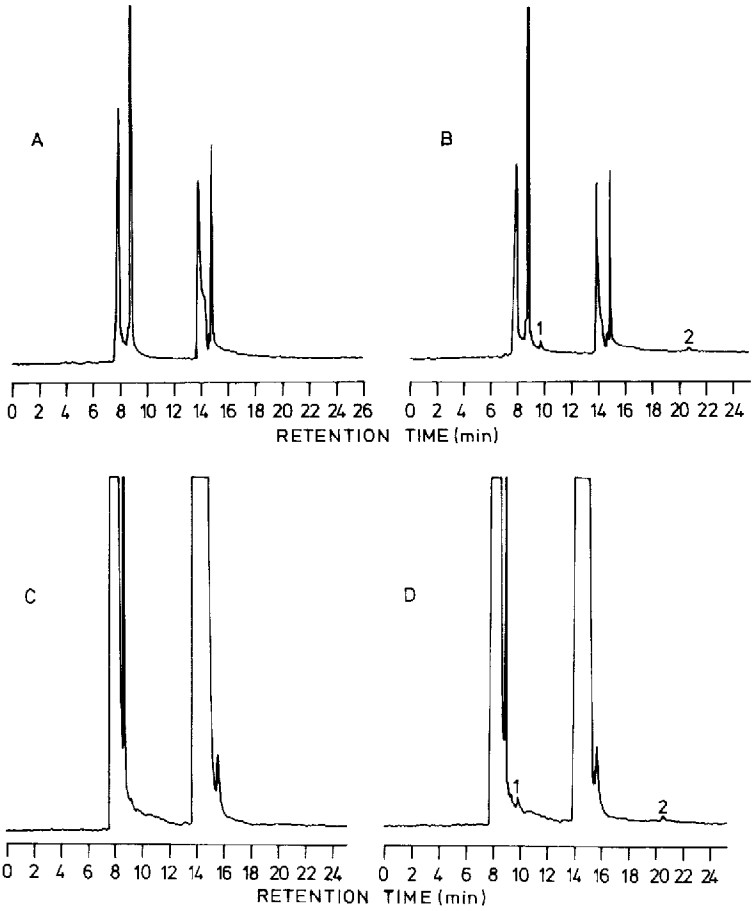

Fig. 6. Chromatograms of (A) blank plasma and (B) plasma from a healthy man, spiked with 10 ng/ml each of α-amanitin and phalloidin; (C) blank plasma and (D) plasma from a geriatric patient, spiked with 10 ng/ml each of α-amanitin and phalloidin. Peaks: 1 = α-amanitin, retention time 9.69 min; 2 = phalloidin, retention time (B) 20.48 min and (D) 20.50 min. Chromatographic conditions: programme A; pre-column S1, RP-8 Spheri-5, particle size 5 μm, 3 cm × 2.1 mm I.D.; analytical column S4, RP-8 Spheri-5, particle size 5 μm, 10 cm × 4.6 mm I.D.; other chromatographic conditions as in Fig. 4.

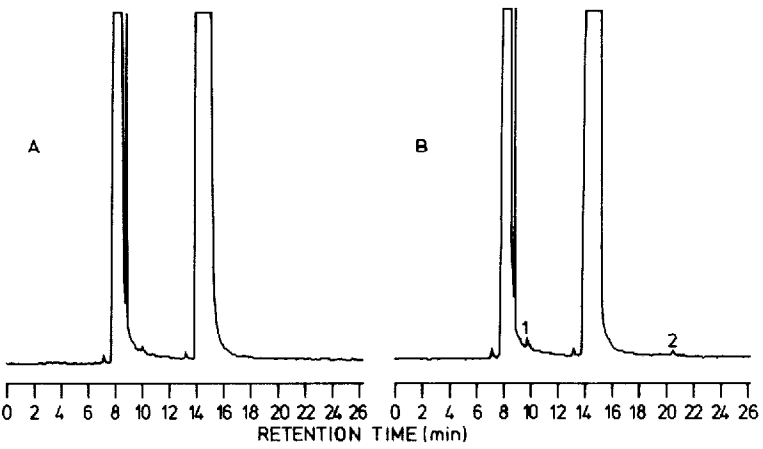

Fig. 7. Chromatograms of (A) plasma suspected to contain toxins of *Amanita phalloides* and (B) patient's plasma spiked with 10 ng/ml each of α-amanitin and phalloidin. Peaks: 1 = α-amanitin, retention time 9.71 min; 2 = phalloidin, retention time 20.50 min. Chromatographic conditions as in Fig. 6.

R.S.D. = 7.76%, $n = 7$; phalloidin, $\bar{x} = 10.11$ ng/ml, R.S.D. = 9.93%, $n = 5$). In order to check for possible interferences by the plasma matrix with the determination of α-amanitin and phalloidin, plasma samples from three different groups were used for the investigations. Typical chromatograms (programme A) for groups 1 and 3 are shown in Fig. 6. The groups were (1) healthy men taking no medicaments, but smokers and coffee drinkers ($n = 2$); (2) healthy women taking no medicaments other than regularly taking estrogen preparations ($n = 3$); and (3) multi-morbid, geriatric patients of both sexes ($n = 5$) undergoing pharmacotherapy with Adalat, Digimerck, Dusodril, Foligan, Fluimucil, Isoptin, Isoket, Lasix and Liquemin.

Childrens' plasma was not used because of the relatively large amounts required; such investigations must be reserved for emergency cases only. Blank plasma samples showed no interferences in the retention time ranges of α-amanitin (9.66–9.76 min) and phalloidin (20.40–20.60 min), as can be seen from Fig. 6, and similarly in chromatograms of group 2 samples (not shown). Silibinin, which is used inter alia in the therapy of α-amanitin intoxication [14], also did not interfere with the two toxins.

Fig. 7A shows the chromatogram (programme A) of a plasma sample taken from a patient with suspected poisoning by *Amanita phalloides* the day before. Fig. 7B shows the chromatogram of this plasma, spiked with α-amanitin and phalloidin (10 ng/ml of each). On the basis of the negative α-amanitin result according to Fig. 7A, the therapeutic treatment with Legalon (Silymarin) and Paspertin (metoclopramid) could be stopped and the patient was discharged from the hospital after one day.

With patients in a hepatic coma, particular analytical problems can arise, the circumvention of which can be demonstrated by the following example. Fig. 8A shows the chromatogram (programme A) of a plasma sample taken from such a patient (A.S., born 27-10-1938; Klinikum Nürnberg, F.R.G.), for whom, inter

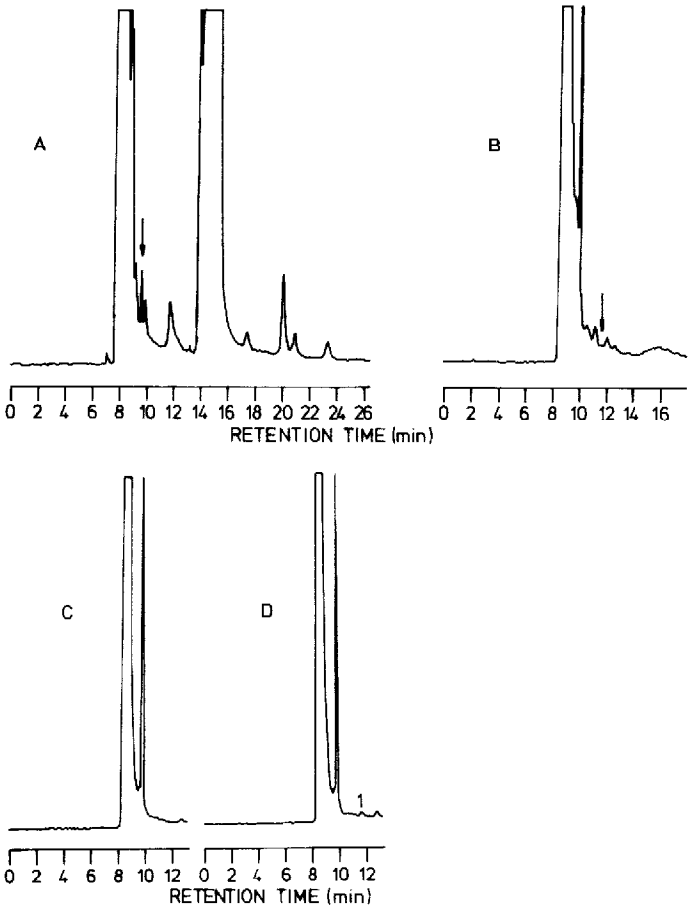

Fig. 8. Chromatograms of (A) plasma obtained from a patient in a hepatic coma, also suspected to contain toxins of *Amanita phalloides*; chromatographic conditions as in Fig. 6; (B) same plasma sample as in (A), analysed by programme B; (C) blank plasma from another patient; (D) same plasma as in (C) spiked with 10 ng/ml α-amanitin. The arrows in (A) and (B) indicate the retention time of α-amanitin. Peak 1 = α-amanitin, retention time 11.5 min. Chromatographic conditions in (B)-(D) as in Fig. 6, except programme B. Analytical columns: S2, RP-8 Spheri-5, particle size 5 μm, 10 cm $\times$ 4.6 mm I.D.; S3, RP-18 Spheri-5, particle size 5 μm, 10 cm $\times$ 4.6 mm I.D.

alia, probable poisoning by *Amanita phalloides* had to be checked. The blood sample had been taken two days before his death (acute liver dystrophy, kidney failure, circulatory failure). At this time the patient had already been treated with Lasix, Augmentan, Nootrop, Zantic, Gastrozepin, Urbason, Kybernin, Maaloxan, Bifiteral, Konakion, Humatin, Liquemin, AS solution, glucose, fructose and Trental. Which of these drugs, which combination of them and which of the metabolites formed from the drugs in the body, or which endogenous substance in this patient had caused the interference in the α-amanitin region was not investigated. This was not even the object of this work. It is of decisive importance that with patients in a hepatic coma interferences with α-amanitin have to be considered in the chromatographic analysis according to programme A.

For the above-mentioned hepatic coma patient, it has now been demonstrated that our method makes it possible to check the analytical results with respect to α-amanitin with the aid of programme B (Fig. 8B). As the amount of the patient's plasma available was no longer sufficient for the analysis of a spiked sample according to programme B, the chromatograms of blank and spiked (10 ng/ml α-amanitin) plasma samples from another patient are shown in Fig. 8C and D. As can be seen from Fig. 8D, the α-amanitin now appears after 11.5 min when programme B is used. In the chromatogram of the plasma sample taken from the liver coma patient (Fig. 8B) no peak is present at this retention time. Therefore, no α-amanitin was detectable at this time in the case history.

CONCLUSION

A rapid (30 min) and sensitive (10 ng/ml in plasma) assay of amanita toxins has been developed as an aid for the diagnosis and therapeutic control of suspected cases of amanita poisoning.

ACKNOWLEDGEMENT

This work was supported by the Wilhelm-Sander-Stiftung. This work is dedicated to the 65th birthday of Professor V. Becker, Institut für Pathologie, Universität Erlangen-Nürnberg.

REFERENCES

1 K. Hruby, K. Lenz, C.D. Moser, J. Bachner and C. Korninger, Wiener Klin. Wochenschr., 91 (1979) 509.
2 R. Fantozzi, F. Ledda, L. Caramelli, F. Moroni, P. Blandina, E. Massini, P. Botti, S. Peruzzi, M. Zorn and P.F. Mannaioni, Klin. Wochenschr., 64 (1986) 38.
3 T. Wieland and H. Faulstich, Crit. Rev. Biochem., 5 (1978) 88.
4 H. Faulstich, Klin. Wochenschr., 57 (1979) 1143.
5 T. Wieland, Chem. Unserer Zeit, 13 (1979) 56.
6 H. Faulstich, H. Trischmann and S. Zobeley, FEBS Lett., 56 (1975) 312.
7 H. Faulstich, S. Zobeley and H. Trischmann, Toxicon, 20 (1982) 913.
8 L. Fiume, C. Busi, G. Campadelli-Fiume and C. Granceschi, Experientia, 37 (1975) 1233.
9 R.Y. Andres, W. Frei, K. Gautschi and D.J. Vonderschmitt, Clin. Chem., 32 (1986) 1751.
10 L. Pastorello, D. Tolentino, M. D'Alterio, R. Paladino, A. Frigerio, N. Bergamo and A. Valli, J. Chromatogr., 233 (1982) 398.
11 F. Jehl, C. Gallion, P. Birckel, A. Jaeger, F. Flesch and R. Ninck, Anal. Biochem., 149 (1983) 35.
12 F. Belliardo and G. Massano, J. Liq. Chromatogr., 6 (1983) 551.
13 G. Caccialanza, C. Gandini and R. Ponci, J. Pharm. Biomed. Anal., 3 (1985) 179.
14 K. Hruby, M. Fuhrmann, G. Csomos and H. Thaler, Wiener Klin. Wochenschr., 95 (1983) 2.