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Automated determination of verapamil and norverapamil in human plasma with on-line coupling of dialysis to high-performance liquid chromatography and fluorometric detection

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Abstract

A fully automated method for the simultaneous determination of verapamil and its main metabolite norverapamil in human plasma is described. This method is based on on-line sample preparation using dialysis followed by clean-up and enrichment of the dialysate on a precolumn and subsequent HPLC analysis with fluorometric detection. All sample handling operations were performed automatically by a sample processor equipped with a robotic arm (ASTED system). The plasma samples were dialysed on a cellulose acetate membrane (cut-off: 15 kD) and the dialysate was purified and enriched on a short pre-column filled with cyanopropyl silica. Before starting dialysis, this trace enrichment column (TEC) was first conditioned with the HPLC mobile phase and then with pH 3.0 acetate buffer. 370 μl of plasma sample spiked with the internal standard (gallopamil) were dialysed in the static-pulsed mode. The solution at the donor side was pH 3.0 acetate buffer containing Triton X-100 while the acceptor solution was made of the same acetate buffer. When dialysis was discontinued, the analytes were desorbed from the TEC by the HPLC mobile phase and transferred to the C_{18} analytical column by means of a switching valve. This mobile phase consisted of a mixture of acetonitrile, pH 3.0 acetate buffer and 2-aminoheptane. The influence of different parameters of the dialysis process on the recovery of verapamil and norverapamil has been studied. The effect of the volume, the aspirating and dispensing flow-rates of the dialysis solution has been investigated. The recoveries of verapamil and norverapamil in plasma were close to 75% and the limits of quantification were 5 ng/ml for both analytes. The method was found to be linear in the concentration range from 5 to 500 ng/ml (r^2: 0.9996 for both analytes). The intra-day and inter-day reproducibilities at a concentration of 100 ng/ml were 2.3% and 5.6% for verapamil and 1.7% and 5.1% for norverapamil, respectively.

Keywords: Dialysis; Sample preparation; Automation; Verapamil; Norverapamil; Gallopamil

1. Introduction

Many high-performance liquid chromatographic (HPLC) methods for the determination of verapamil and its N-demethylated metabolite, norverapamil, in biological fluids have been reported [1–9]. They involve ultraviolet detection [2] or more frequently fluorescence detection [1,3–9] owing to the native fluorescence properties of these compounds.

Generally, the sample preparation consists of a liquid–liquid extraction (LLE) of the analytes from plasma followed in most cases by a back-extraction into an acidic aqueous solution [3–5,7,8]. These techniques are often tedious and time-consuming and an interesting alternative is the isolation of the analytes by liquid–solid or solid-phase extraction

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(SPE) using column switching or disposable extraction cartridges (DECs) [8–11].

Another fully automated approach consists of using dialysis as sample preparation step prior to HPLC. This on-line technique combines dialysis, clean-up and trace enrichment, making it possible to load untreated plasma samples directly onto the HPLC autosampler [12,13]. Only low-molecular-mass compounds can pass through the cellulose acetate membrane (molecular mass cut-off: $15 \cdot 10^3$) while proteins and particles are removed. The dialysate is then directed to a trace enrichment column (TEC) where the compounds of interest are concentrated while more polar solutes are eliminated. By means of a switching valve, the analytes are desorbed from the TEC by the HPLC mobile phase and transferred to the analytical column. The ASTED (automated sequential trace enrichment of dialysate) system is able to perform automatically all sample handling operations. This technique has already been applied to plasma, serum, whole blood and tissue homogenate samples [14–21]. The purpose of this paper is to describe such a fully automated method, developed for the determination of verapamil and its main metabolite, norverapamil, in plasma. The method involves on-line sample preparation using dialysis as a sample preparation followed by precolumn clean-up and enrichment of the dialysate and subsequent HPLC analysis using fluorometric detection. The influence of different parameters of the dialysis procedure on the recoveries of verapamil and norverapamil has been studied. The volume, the aspirating and dispensing flow-rates of the dialysis liquid were the main parameters investigated. Moreover, the effect of the nature of the sorbent used in the TEC was also investigated. The breakthrough volumes of the three compounds of interest were determined for both sorbents tested. Finally, the parameters studied were optimized with respect to analyte recoveries and the method developed was validated.

2. Experimental

2.1. Chemical and reagents

R,S-Verapamil, its N-demethylated derivative, norverapamil and gallopamil (internal standard) were kindly supplied by S.M.B. Pharmaceuticals (Brussels, Belgium) and were used without further purification.

Sodium acetate, glacial acetic acid, Triton X-100 and sodium azide were of analytical grade from Merck (Darmstadt, Germany). 2-Aminoheptane was purchased from Aldrich (Gillingham, UK) and was distilled twice before use [9]. Methanol was of HPLC grade from Janssen (Geel, Belgium). Acetonitrile was of HPLC grade from Merck (LiChroSolv). Water used in all experiments was of Milli-Q quality (Millipore, Bedford, MA, USA).

The LiChroCart analytical column was prepacked with Superspher 100 RP-18 (particle size: 4 μm) and the LiChroCart guard column with LiChrospher 100 RP-18 (5 μm) from Merck. The sorbents used in the trace enrichment columns were Hypersil RP-18 (10 μm) (prepacked TECs from Gilson Medical Electronics) and Nucleosil CN (30 μm) from Macherey-Nagel (Düren, Germany).

2.2. Apparatus

The chromatographic instrumentation included an ASTED system from Gilson (Villiers-le-Bel, France) and a model 305 pump (Gilson). A model Dynamax UV-1 variable-wavelength UV–visible absorbance detector (Rainin, Woburn, MA, USA) was used for the development of the dialysis step while a model RF-10A fluorescence detector from Shimadzu (Kyoto, Japan) was used for method validation.

The UV detector was set at a wavelength of 278 nm while for fluorescence detection, an excitation wavelength of 275 nm and an emission wavelength of 310 nm were selected [8,9,22].

A schematic representation of the ASTED system is shown in Fig. 1. It consists of an automatic sampling injector, two 1-ml model 401 diluters and a dialysis cell of 370 μl. The dialysis cell contained a cellulose acetate dialysis membrane (Cuprophan) with a molecular mass cut-off of $15 \cdot 10^3$. Two kinds of TECs prepacked either with cyanopropyl silica (11×4 mm I.D., particle size: 30 μm) from Macherey–Nagel or with octadecyl silica (10×4 mm I.D., particle size 10 μm) from Gilson were tested. A six-port valve (model 7010, Rheodyne, Berkeley, CA, USA) was used to connect the TEC to the acceptor channel of the dialysis cell or to the analytical column.

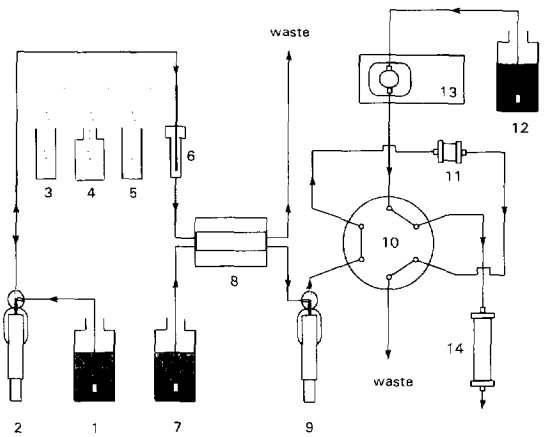

Fig. 1. Schematic representation of the ASTED system. 1: Priming solution; 2: dilutor 1; 3: sample vial; 4: internal standard; 5: mixing vial; 6: injector; 7: acceptor solution; 8: dialysis bloc; 9: dilutor 2; 10: injection valve; 11: trace enrichment column; 12: HPLC mobile phase; 13: HPLC pump; 14: analytical column.

A Manu-Cart system which consisted of a LiChroCart analytical column (125×4 mm I.D.) and a short LiChroCart guard column (4×4 mm I.D.) from Merck were thermostated at 35±0.1°C in a model 20 B/VC Julabo waterbath (Seelbach, Germany).

An IBM compatible computer (PC-AT; CPU type 80486) equipped with the "715 HPLC System Controller" and the "722 Keypad" softwares from Gilson was used to control the HPLC and the ASTED systems, respectively.

2.3. Chromatographic conditions

The mobile phase consisted of a mixture of acetonitrile–2-aminoheptane–0.01 *M* sodium acetate buffer adjusted to pH 3.0 with acetic acid (25:0.5:75, v/v/v). Before use, the mobile phase was degassed for 15 min in an ultrasonic bath. The flow-rate was 0.9 ml/min.

2.4. Dialysis conditions

The priming solution for the donor side of the dialysis cell consisted of 0.01 *M* acetate buffer containing 0.01% (v/v) of Triton X-100 and 50 mg/l of sodium azide. The acceptor solution consisted of the same acetate buffer, containing 50 mg/l of sodium azide.

2.5. Standard solutions

2.5.1. Solutions used for the determination of breakthrough volumes

Stock solutions of verapamil (V), norverapamil (NV) and gallopamil (I.S.) were prepared by dissolving 25 mg of each compound in 25 ml of pH 3.0 acetate buffer. The three solutions used for the determination of breakthrough volumes were prepared by diluting 0.5 ml of stock solution of NV, V or I.S. to a final volume of 300 ml (1.67 μg/ml).

2.5.2. Solutions used for method development

Stock solutions of verapamil, norverapamil and gallopamil (I.S.) were prepared by dissolving 25 mg of each compound in 25 ml of methanol. The solutions used were prepared by diluting with water the stock solutions of norverapamil and verapamil to obtain a concentration of 1 μg/ml for each component. In the same way, two separate solutions of NV and V were diluted with water to a concentration of 10 μg/ml. Plasma samples (1.6 ml) were spiked with 200 μl of each solution for selectivity studies. The stock solution of gallopamil (I.S.) was diluted with water to reach a final concentration of 20 μg/ml.

2.5.3. Solutions used for method validation

Two mixed solutions of norverapamil and verapamil were prepared by diluting a stock solution of norverapamil and verapamil with water to obtain concentrations of 10 μg/ml and 1 μg/ml for both analytes, respectively. These two solutions were used to spike plasma samples (2 ml) for calibration curves (from 5 to 500 ng/ml). The stock solution of gallopamil was diluted with water to obtain a final concentration of 1.2 μg/ml.

2.6. Sample preparation

After centrifugation of the plasma sample at 6000 rpm (~6000 *g*) for 10 min, a 500 μl volume of plasma was transferred into a vial placed on the appropriate rack of the ASTED XL system. All the sample handling operations were then executed automatically by the sample processor.

- *Washing*: the donor channel of the dialyser was washed with 2.0 ml of pH 3.0 acetate buffer

containing Triton X-100 and sodium azide (flow-rate: 3.0 ml/min) while the acceptor channel was washed with 2.0 ml of the same buffer containing sodium azide.

- *TEC conditioning*: the trace enrichment column was first conditioned twice with 0.995 ml of mobile phase (flow-rate: 3.0 ml/min) and then by dispensing 0.995 ml of acetate buffer pH 3.0 containing sodium azide.
- *Addition of internal standard*: a 50 μl volume of gallopamil was added to 450 μl of plasma sample and mixed by bubbling.
- *Dialysis*: 370 μl of plasma were aspirated (1.0 ml/min) by the autosampler needle (diluter 1) and dispensed in the donor channel of the dialysis cell (1.0 ml/min). The sample was then held static while 9 ml of the acceptor solution were pumped through the acceptor channel in 1 ml-pulses (diluter 2; 1.0 ml/min). The diluter 2 dispensed simultaneously the dialysate onto the TEC in the pulse mode (1.0 ml/min).
- *Injection and washing*: when dialysis was discontinued, the analytes retained on the TEC were eluted in the back-flush mode with the mobile phase by rotation of the switching valve for 2 min. After elution of the analytes, the TEC was conditioned and the preparation of a new sample was started while the previous one was analysed by HPLC.

3. Results and discussion

3.1. Chromatographic separation

The mobile phase mentioned in the automated SPE–HPLC procedure developed earlier [8,9] was first used for the separation of verapamil, norverapamil and gallopamil. However, due to the use of a slightly different analytical column in this work, the concentration of the organic modifier present in the mobile phase had to be adjusted in order to obtain a good separation of the three compounds. The acetonitrile content of the mobile phase selected in the dialysis–HPLC procedure was finally 5% lower than the one used in the SPE–HPLC procedure.

3.2. Determination of breakthrough volumes

The breakthrough volume of an analyte corresponds to the volume of liquid needed for the elution of this compound from a column. It depends on the composition of the liquid in which the compound is dissolved and on its flow-rate, and also on the type of solid phase and the dimensions of the column.

The measurement of breakthrough volumes permits the determination of the maximum volumes of liquid that can be used for the dialysis of samples. Fig. 2 illustrates the monitoring of the breakthrough

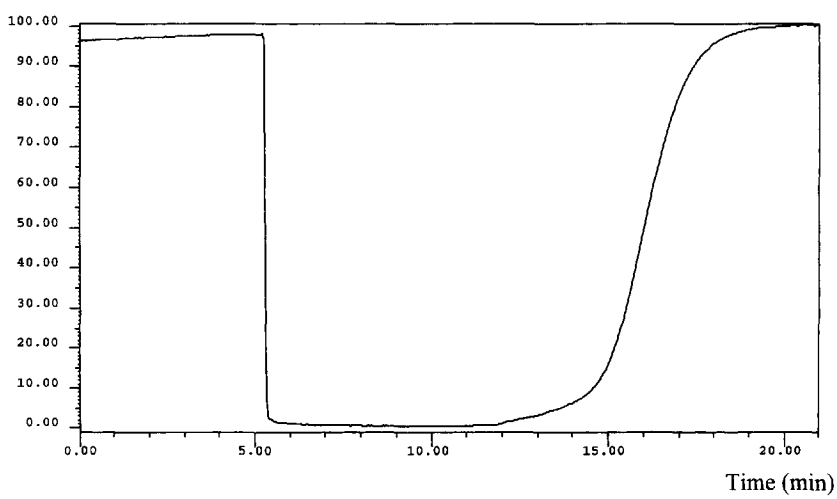

Fig. 2. Monitoring of the breakthrough volume of verapamil. Solution: 1.7 μg/ml verapamil in pH 3.0 acetate buffer; TEC: Nucleosil CN (30 μm, 11×4 mm I.D.); Detection: UV at 278 nm; Flow-rate: 0.9 ml/min.

volume of verapamil on a TEC filled with cyanopropyl silica.

The breakthrough volume was determined by monitoring the UV absorbance of a solution of the analyte in the dialysis liquid. An abrupt decrease of absorbance was first observed, due to the adsorption of the analyte to the TEC. The breakthrough volume of the analyte was reached when the absorbance was found to increase up to a fairly constant value (plateau). The breakthrough volumes of the three compounds of interest (NV, V and I.S.) were determined using both types of TEC (Table 1).

3.3. Dialysis

The addition of the detergent Triton X-100 was found to improve the reproducibility of the results [23]. Sodium azide was added to prevent microbial proliferation in the dialysis cell.

The extraction cartridges used in the SPE procedure developed by Hubert et al. [8,9] were filled with a cyanopropyl silica support (mean particle size: 40 μm). By analogy, TECs filled with the same kind of CN phase were tested in this work. Fig. 3 shows a comparison of chromatograms obtained with blank plasma samples by using TECs filled with octadecylsilica and with cyanopropyl silica. The chromatograms obtained with the C_{18} phase shows endogenous peaks in the retention time range of norverapamil and verapamil. However, with the CN phase, no interferences were observed in the same retention range. On the other hand, the endogenous peak observed around 20 min was obviously less pronounced when the CN TEC was used. Therefore, this support was selected as TEC sorbent for the compounds of interest.

The acceptor solution was passed through the dialyser in 1.0-ml pulses. Fig. 4 shows the dialysis recovery for verapamil, norverapamil and gallopamil in aqueous solutions when the volume of the acceptor solution was varied from 1 to 17 ml. The aspirating and dispensing flow-rates were both kept at a constant level of 1.0 ml/min. Dialysis recoveries were expressed in terms of relative recoveries (%), calculated by comparing analyte peak areas found after dialysis to those obtained when aqueous solutions of the drugs at the same concentration were injected directly onto the TEC.

An increase of the volume of acceptor solution from 1 to 9 ml gave rise to better relative recoveries for the three analytes. The increase was more pronounced for gallopamil (I.S.) than for NV and V. The maximum relative recovery of verapamil and norverapamil were observed when 10 ml of dialysis liquid were used. However, for the I.S., the maximum was reached by using 9 ml of acceptor solution. When using larger volumes than 10 ml, the dialysis recoveries of the three compounds were decreased. This is probably due to the elution of the analytes from the TEC by the acceptor solution itself, as illustrated by Figs. 2 and 4.

The same investigations were made with plasma samples spiked with norverapamil, verapamil and gallopamil. The relative recoveries of the three compounds were determined with increasing volume of the acceptor solution. Fig. 4 shows a similar behaviour of the relative recoveries. The recoveries observed for aqueous solutions and for spiked plasma samples were not significantly different. This seems to demonstrate that the optimisation of the dialysis process can be performed by using aqueous solutions of the analytes. Finally, a 9-ml volume of acceptor solution was selected because it gave the maximum dialysis recovery for norverapamil and verapamil.

The influence of the aspirating flow-rate of the dialysis liquid on the analyte recoveries was also investigated (Table 2). Even if an increase of the relative recoveries of both analytes was observed when this flow-rate was decreased, it is not recommended to choose too low aspirating flow-rates because the sample preparation time can then become very long. An aspirating flow-rate of 1.0 ml/min was finally found to be a good compromise. Under these conditions, the mean dialysis recoveries for aqueous solutions were 68.5% for verapamil,

Table 1
Breakthrough volumes of NV, V and I.S. on two types of TEC

	TEC RP-18(ml)	TEC-CN(ml)
Norverapamil	>60	9.2
Verapamil	>60	9.7
Gallopamil	>60	9.0

Solution: 1.7 μg/ml of analyte in pH 3.0 acetate buffer; Detection: 278 nm; TEC: Hypersil RP-18 (10 μm, 10×4 mm I.D.) or Nucleosil CN (30 μm, 11×4 mm I.D.).

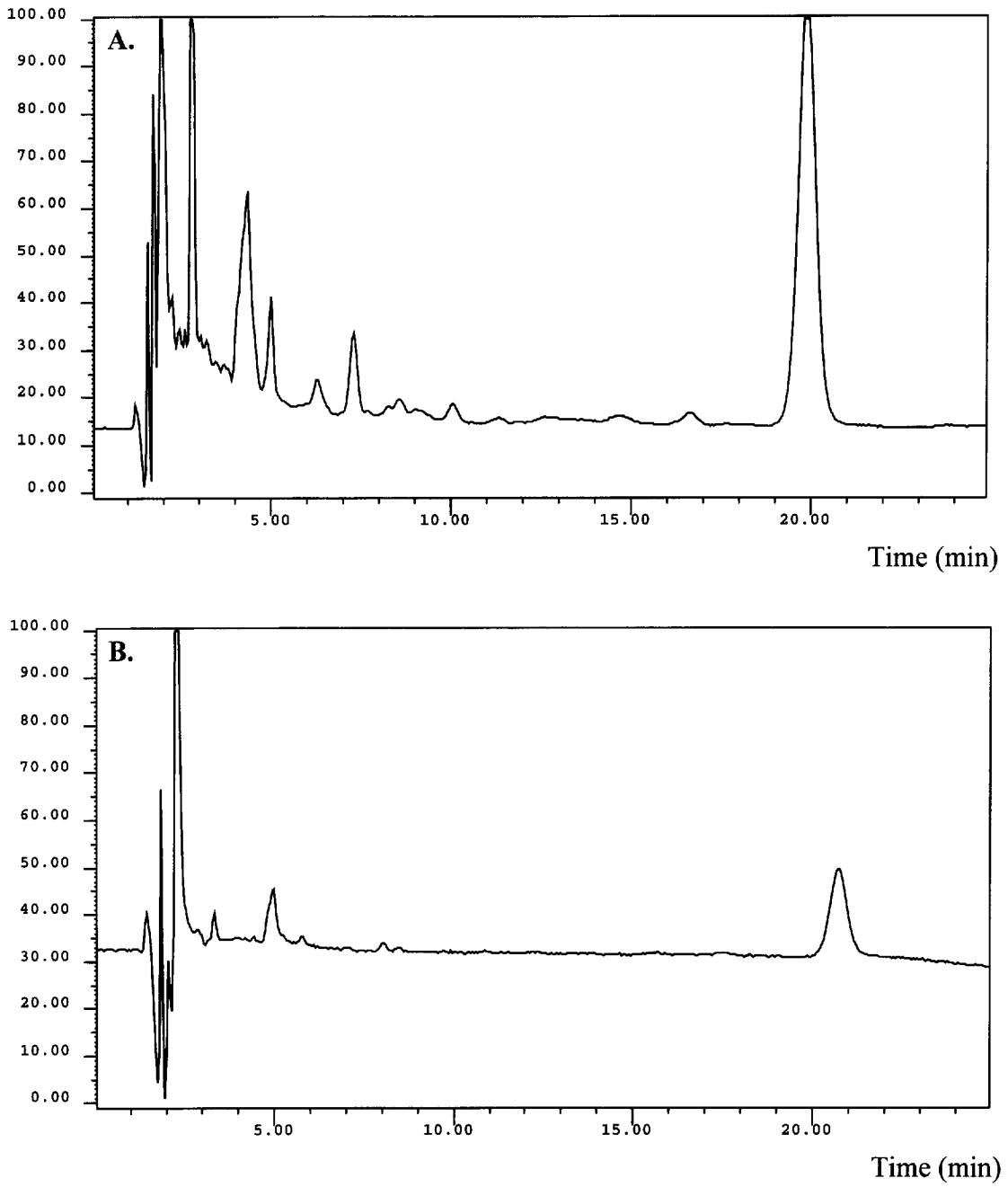

Fig. 3. Influence of the TEC sorbent on selectivity. TEC: (A) Hypersil RP-18 (10 μm, 10×4 mm I.D.) or (B) Nucleosil CN (30 μm, 11×4 mm I.D.); Dialysis mode: static/pulsed; Aspirating flow-rate: 1.0 ml/min; Dispensing flow-rate: 1.0 ml/min; Dialyser: 370 μl for donor channel and 650 μl for acceptor channel; Dialysis liquid: pH 3.0 acetate buffer; Sample volume: 370 μl of blank plasma; Detection: UV at 278 nm.

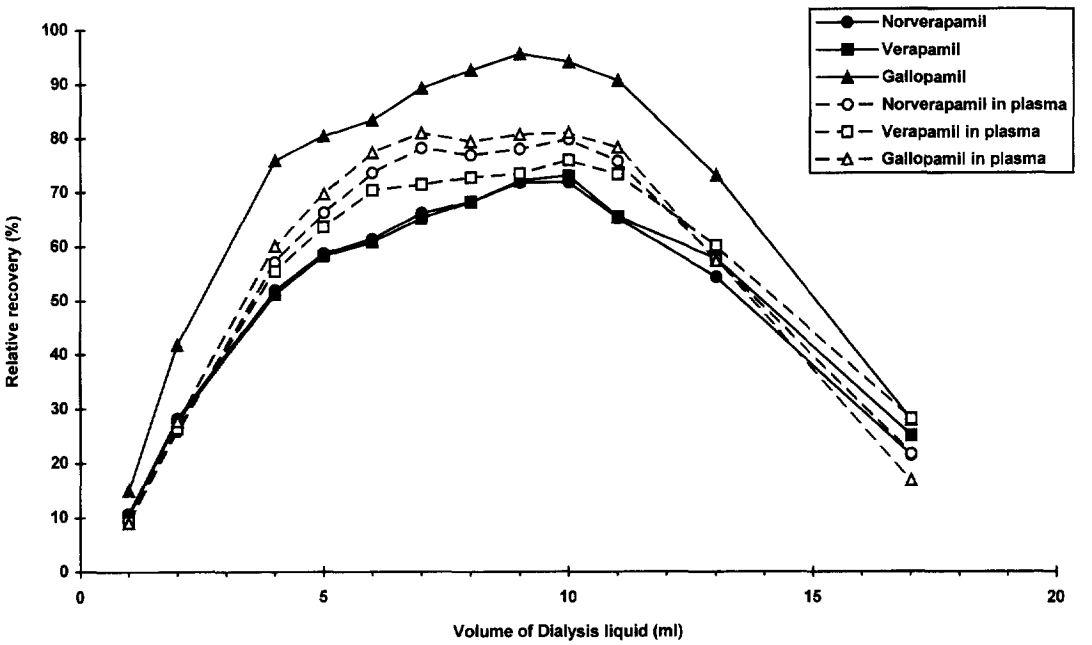

Fig. 4. Influence of the volume of dialysis liquid on dialysis recovery, using aqueous solutions of the analytes and spiked plasma samples. Dialysis mode: static/pulsed; Aspirating flow-rate: 1.0 ml/min; Dispensing flow-rate: 1.0 ml/min; Dialyser: 370 μl for donor channel and 650 μl for acceptor channel; Dialysis liquid: pH 3.0 acetate buffer; Sample volume: 370 μl of solution; TEC: Nucleosil CN (30 μm), 11×4 mm I.D.

Table 2
Influence of the aspirating and dispensing flow-rates on dialysis recovery

Aspirating flow-rate (ml/min)	Dispensing flow-rate (ml/min)	Relative recovery (%) (n=3)		
		NV	V	I.S.
0.5	1.0	70	72	98
1.0	1.0	66	66	94
2.0	1.0	63	61	90
3.0	1.0	63	62	88
4.0	1.0	62	62	89
5.0	1.0	63	63	89
1.0	0.5	74	75	96
1.0	1.0	71	72	90
1.0	2.0	67	69	84
1.0	3.0	64	65	81
1.0	4.0	62	64	76
1.0	5.0	47	47	59

Dialysis mode: static/pulsed; Dialysis liquid: 10 ml of pH 3.0 acetate buffer; Dialyser: donor channel=370 μl, acceptor channel=650 μl; Sample volume loading: 370 μl of aqueous solution; TEC: Nucleosil CN (30 μm), 11×4 mm I.D.; Sample solution: verapamil and norverapamil in water (1 μg/ml).

69.0% for norverapamil and 92.0% for gallopamil, respectively.

Table 2 also shows the influence of the dispensing flow-rate of the dialysate onto the TEC on analyte recoveries. High dispensing flow-rates (5 ml/min) seem to give rise to lower recoveries. However, it should be noted that the influence of the aspirating and dispensing flow-rates was not as important as the volume of dialysis liquid, which seems to be the most important parameter to optimize in the dialysis process. Under these final conditions, fairly good relative recoveries of about 75% were obtained (Table 3).

3.4. Method validation

3.4.1. Selectivity

A typical chromatographic trace of a plasma extract containing verapamil, norverapamil and gallopamil is shown in Fig. 5. Under the conditions selected for the HPLC separation, the mean capacity

Table 3
Absolute and relative recoveries of the analytes (concentration: 100 ng/ml)

	Norverapamil	Verapamil	Gallopamil
Relative recoveries	75.2%	73.4%	80.7%
Absolute recoveries	76.1%	73.2%	80.9%

ratios (k') of norverapamil, verapamil and gallopamil were 6.8, 7.7 and 9.7. respectively ($n=20$).

No endogenous sources of interference were observed at the retention times of the analytes. Typical chromatograms obtained with a blank plasma and with a spiked plasma containing 5 ng/ml of verapamil and norverapamil are presented in Fig. 5.

3.4.2. Absolute recovery

Table 3 gives relative and absolute recoveries of the analytes at a 100 ng/ml concentration. The absolute recoveries for norverapamil and verapamil were also close to 75%. The relative recoveries were calculated by comparing peak heights obtained from freshly prepared sample extracts with those found by injection on the TEC of aqueous standard solutions at the same concentration, using the same autosampler. The absolute recoveries were calculated by comparing peak heights obtained from freshly prepared sample extracts with those found by direct injection of aqueous standard solutions at the same concentration, using the same autosampler but equipped with a sample loop of 100 μl instead of the trace enrichment column [24]. The similarity of the values obtained for relative and absolute recoveries leads to the conclusion that the totality of the analytes of interest are eluted from the TEC.

3.4.3. Linearity

The calibration curves were obtained in the range 5–500 ng/ml ($n=7$), since the therapeutic plasma levels of verapamil did not exceed 500 ng/ml [25]. The linear regression analysis of the peak height ratio (y) versus the concentration (x) in ng/ml gave the following equations (concentration range 5–500 ng/ml; $n=7$):

norverapamil: $y=0.0345x-0.0051$ $r^2=0.9996$
verapamil: $y=0.0303x-0.0089$ $r^2=0.9996$

The linearity of the calibration curves is demonstrated by the good determination coefficients (r^2) obtained for the two regression lines.

3.4.4. Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were determined as the concentrations of substance giving rise to signal-to-noise ratios of 3 and 10, respectively. For the analytes studied, the LODs were found to be 1.3 for verapamil and 1.4 ng/ml for norverapamil, respectively. The LOQs were 4.3 and 4.8 ng/ml, respectively.

3.4.5. Reproducibility

As shown in Table 4, the precision of the automated bioanalytical method was estimated by measuring the intra-day and inter-day reproducibilities of the analytes at seven concentration levels, ranging from 5 to 500 ng/ml. The mean values for intra-day and inter-day reproducibilities were 2.4% and 7.9% for verapamil and 2.6% and 7.3% for norverapamil, respectively.

3.4.6. Accuracy

The overall accuracy of the procedure was assessed by plotting the graph of the amount found versus the amount applied in spiked plasma samples at 3 concentrations levels ($n=6$) ranging from 5 to 500 ng/ml ($r^2=0.9996$ for both analytes).

The t-tests showed that the slope of the line was not significantly different from unity ($t_{cal}=0.32$ for verapamil and 1.07 for norverapamil) and that the line passed through the origin ($t_{cal}=0.46$ for norverapamil and 0.57 verapamil). All t-values correspond to $p>0.05$. The automated HPLC procedure for the determination of verapamil and norverapamil in human plasma using dialysis as sample preparation can thus be considered as accurate and linear within the concentration range investigated.

Acknowledgments

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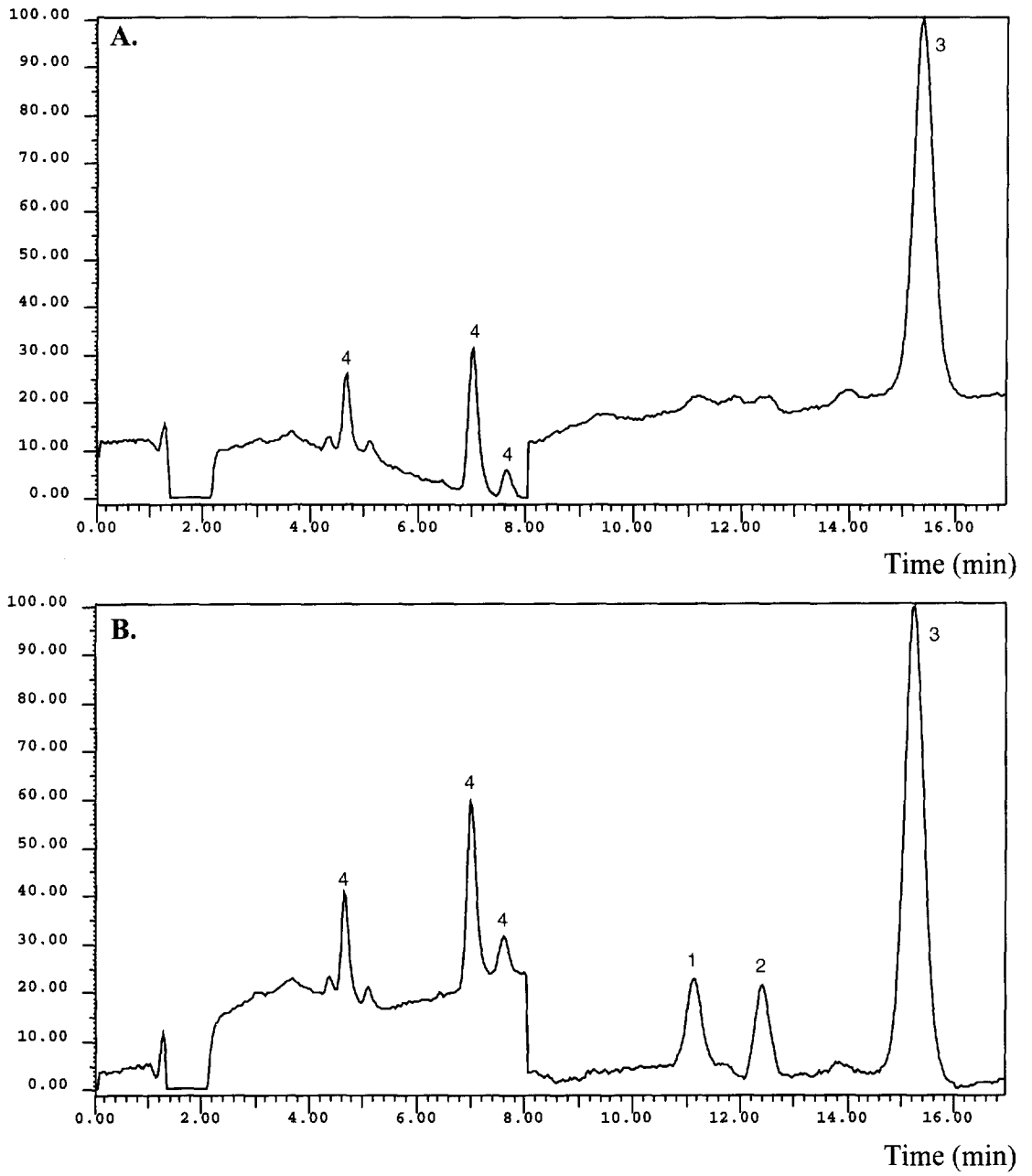

Fig. 5. Typical chromatograms obtained by using dialysis coupled on-line to HPLC. A: Chromatogram of blank plasma. B: Chromatogram of plasma spiked with norverapamil and verapamil (5 ng/ml). Chromatographic and dialysis conditions as described in Sections 2.3 and 2.4, with fluorescence detection (excitation wavelength: 275 nm; emission wavelength: 310 nm). Peaks: 1. Norverapamil (5 ng/ml); 2. Verapamil (5 ng/ml); 3. Gallopamil (I.S.); 4. Endogenous peak.

Table 4
Reproducibility of the automated dialysis–HPLC method

Reproducibility	n	Concentration (ng/ml)	Verapamil (RSD, %)	Norverapamil (RSD, %)
Within-day	6	5	6.7	5.1
	3	10	2.7	3.5
	3	25	1.4	3.8
	3	50	0.7	1.8
	6	100	2.3	1.7
	3	250	1.4	0.9
	6	500	1.5	1.4
		Mean (n=7)	2.4	2.6
Between-day	6	5	11.2	7.5
	3	10	8.5	8.9
	3	25	8.5	8.0
	3	50	13.7	14.8
	6	100	5.6	5.1
	3	250	4.0	4.0
	6	500	4.5	2.7
		Mean (n=7)	7.9	7.3

RSD: Relative standard deviation.

References

[1] S.C.J. Cole, R.J. Flanagan, A. Johnston and D.W. Holt, J. Chromatogr., 218 (1981) 621.
[2] C. Giacchetti, P. Poletti and G. Zanolo, J. High Resolut. Chromatogr., 10 (1987) 654.
[3] S.R. Harapat and R.E. Kates, J. Chromatogr., 170 (1979) 385.
[4] S.R. Harapat and R.E. Kates, J. Chromatogr., 181 (1980) 484.
[5] T.M. Jaouni, M.B. Leon, D.R. Rosing and H.M. Fales, J. Chromatogr., 182 (1980) 473.
[6] M. Kuwada, T. Tateyama and J. Tsutsumi, J. Chromatogr., 222 (1981) 507.
[7] Y.K. Piotrovskii, D.O. Rumianisev and V.I. Metelitsa, J. Chomatogr., 275 (1983) 195.
[8] Ph. Hubert, P. Chiap, A. Ceccato, I. Bechet, R. Sibenaler-Dechamps, P. Maes and J. Crommen, J. Pharm. Biomed. Anal., 10 (1992) 937.
[9] Ph. Hubert and J. Crommen, J. Liq. Chromatogr., 17 (1994) 2147.
[10] D. Westerlund, Chromatographia, 24 (1987) 155.
[11] H. Lingeman, R.D. McDowall and U.A.Th. Brinkman, Trends Anal. Chem., 10 (1991) 48.
[12] D.C. Turnell, T.D.H. Cooper, B. Green, G. Hughes and D.J. Wright, Clin. Chem., 34 (1988) 1816.
[13] N.C. van de Merbel and U.A.Th. Brinkman, Trends Anal. Chem., 12 (1993) 249.
[14] T. Agasøster and K.E. Rasmussen, J. Chromatogr., 564 (1991) 171.
[15] T. Agasøster and K.E. Rasmussen, J. Chromatogr., 570 (1991) 99.
[16] A.T. Andresen, P.B. Jacobsen and K.E. Rasmussen, J. Chromatogr., 575 (1992) 93.
[17] A.T. Andresen, K.E. Rasmussen and H.E. Rugstad, J. Chromatogr., 621 (1993) 189.
[18] J.D.H. Cooper, D.C. Turnell, B. Green and F. Verillon, J. Chromatogr., 456 (1988) 53.
[19] N.C. Van de Merbel, J.M. Teule, H. Lingeman and U.A.Th. Brinkman, J. Pharm. Biomed. Anal., 10 (1992) 225.
[20] N.M.L. Aerts, N.M.J. Beck and U.A.Th. Brinkman, J. Chromatogr., 500 (1990) 453.
[21] T. Agasøster and K.E. Rasmussen, J. Pharm. Biomed. Anal., 10 (1992) 349.
[22] R.G. McAllister, T.G. Tan and D.W.A. Bourne, J. Pharm. Sci., 68 (1979) 574.
[23] M. Krogh, A.S. Christophersen and K.E. Rasmussen, J. Chromatogr., 621 (1993) 41.
[24] A.R. Buick, M.V. Doig, S.C. Jeal, G.S. Land and R.D. MacDowall, J. Pharm. Biomed. Anal., 8 (1990) 629.
[25] B.N. Singh, G. Ellrodt and T. Peter, Drugs, 15 (1978) 169.