ELSEVIER

Journal of Chromatography B, 749 (2000) 135–142

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

On-line dialysis and quantitative high-performance liquid chromatography analysis of iodixanol in human, rat and monkey plasma

Petter Balke Jacobsen*

Research and Development, Nycomed Imaging AS, P.O. Box 4220 Torshov, N-0401 Oslo, Norway

Received 28 February 2000; received in revised form 10 July 2000; accepted 10 July 2000

Abstract

A fully automated HPLC method for analysing the non-ionic X-ray contrast agent iodixanol in plasma samples, using on-line dialysis for sample preparation, was developed. Optimal conditions were obtained with a static dialysis donor solution of 110 μl and 4 ml of recipient solution (dialysate) pulsed onto a trace enrichment column, giving maximum 55% dialysis efficiency in less than the chromatographic run time of 20 min. Hence, one sample could be dialysed during the analysis of the previous. The maximum load of iodixanol on the trace enrichment column was 3.75 mg. Validation showed that the method was selective for iodixanol, sensitive down to 84 pmol/ml and had a high precision over a linear range up to 320 nmol/ml. Although developed for iodixanol, the method can easily be modified and applied to other substances with similar properties, i.e., substances having low protein binding and high water solubility, but strong enough stationary phase affinity to be retained by an appropriate trace enrichment column.

Keywords: On-line dialysis; Iodixanol

1. Introduction

While the analytical separation principle per se and the detection often are of less concern due to high precision equipment and advanced technology, the sample pre-treatment procedures of bioanalytical work may bring in several potential sources of errors. With multiple steps of manual handling like filtration, dilution, precipitation, extraction, centrifugation, the risk of introducing errors are high, and a propagation of these errors may give serious deviations of the results.

The automated on-line dialysis system ASTED (automated sequential trace enrichment of dialysates) was developed in order to eliminate manual sample handling for the analysis of water-soluble substances and for measuring free drug concentration in plasma and serum. The system combines sample dialysis with column switching for effective analyte clean-up and was originally designed for on-line coupling to high-performance liquid chromatography (HPLC) [1,2]. Later other techniques like gas chromatography (GC) and GC–mass spectrometry (MS) [3,4], capillary electrophoresis [5] and thermospray tandem MS [6] have been successfully connected to this sample clean-up system. On-line dialysis has been applied to analysis of several drugs and substances,

*Tel.: +47-2318-5502; fax: +47-2318-6008.
E-mail address: pbj@nycomed.com (P.B. Jacobsen).

0378-4347/00/$ – see front matter
PII: S0378-4347(00)00405-9

including analysis of the non-ionic X-ray contrast agent iopentol in whole blood and plasma [7].

The analysis of biological samples containing water-soluble iodinated X-ray contrast agents, including the dimeric non-ionic agent iodixanol (Visipaque), normally involves traditional sample handling steps such as filtration, acidic or organic protein precipitation and centrifugation [8,9]. In addition to the possible introduction of errors, the manual sample handling is time consuming and occupies manpower. Automated sample handling is therefore highly to be preferred if possible to establish. This paper presents a fully automated method for quantitative analysis of iodixanol in plasma using reversed-phase HPLC with on-line dialysis and trace enrichment for sample preparation.

2. Experimental

2.1. Materials and reagents

Iodixanol (molecular mass=1550) was obtained from Nycomed Imaging (Oslo, Norway). Acetonitrile and methanol were of HPLC-grade (Merck, Darmstadt, Germany). Water was purified by reversed osmosis and a Milli-Q system (Millipore, MA, USA). All other chemicals were of analytical-reagent grade. Human plasma with citrate as anticoagulant was obtained from The Red Cross Blood Centre (Oslo, Norway). Animal plasma was obtained from untreated Wistar rats and untreated Cynomolgus monkeys, with EDTA as anticoagulant.

2.2. Samples

A stock solution of 50 mg/ml iodixanol in water was used for the preparation of both the control samples and the calibration samples. Control samples were prepared from human, rat and monkey plasma, spiked with iodixanol (stock solution) to give the final concentrations 0.5, 5.0, 50.0 and 500.0 μg/ml. Enough samples for all validation experiments were prepared and immediately frozen at −20°C in aliquots of 0.5 ml. Calibration samples were prepared from human plasma only. By validating the methods using human calibration samples regardless of the species to be analysed, the problems with low availability of plasma from the actual species and unnecessary sacrificing of untreated animals in preclinical studies are avoided. Plasma was spiked with iodixanol (stock solution) to give the final concentrations 0.1, 1.0, 10.0, 100.0 and 1000.0 μg/ml, and the samples were frozen at −20°C in aliquots of 0.5 ml.

2.3. HPLC equipment and procedure

The HPLC system consisted of an SP8800 ternary pump, an SP 8500 dynamic mixer, an SP8450 UV–Vis detector (Spectra Physics/Thermo Separation Products, Freemont, CA, USA) and an on-line dialysis unit (described separately below). The sample rack temperature was 2–8°C, cooled by a MGW Lauda RMT 6 thermostat water bath (Dr. R. Wobser, Lauda-Königshofen, Germany). The analytical column was a Brownlee OD-5A, Spheri-5, RP-18, 5 μm, 250×4.6 mm operated at ambient temperature, with a Brownlee RP-18 Newguard, 7 μm, 15×3.2 mm pre-column (Applied Biosystems, San Jose, CA, USA). The mobile phase was isocratic acetonitrile in water with a flow-rate of 1 ml/min. The acetonitrile concentration was 9% (v/v) or close to this, depending on any small adjustments necessary to obtain peak separation from endogenous plasma compounds. The detection wavelength was 244 nm, which is the approximate wavelength maximum for iodixanol in this mobile phase.

2.4. On-line dialysis equipment and procedure

The ASTED system (Gilson Medical Electronics, Villiers-le-Bel, France) consisted of a Gilson 231 sample injector equipped with a Rheodyne 7010 injection valve (Rheodyne, Berkley, CA, USA), two Gilson 401 dilutors (1 ml syringe pumps), a dialyser unit with 100 μl donor channel volume and 175 μl recipient channel volume, a cellulose (Cuprophane) dialysis membrane with a molecular mass cut-off at 15 000, a Gilson Prelute (5×1.6 mm Hypersil ODS 10 μm) trace enrichment column (TEC) replacing the sample loop on the injection valve, and Gilson ASTED software v. 1.21.

The ASTED XL system (a newer version of the ASTED system) consisted of a Gilson 233B sample injector equipped with two Rheodyne 7010 injection

valves and two Gilson 401 dilutors. The dialyser unit, the dialysis membrane and the TEC were the same as for the ASTED system. The software was Gilson 722 v. 1.03.

The principle of both systems is that varying amounts of sample can be introduced into the dialyser donor channel, and kept static or pulsed through, by the Gilson 401 dilutor. A recipient solvent is then placed into the dialyser recipient channel and either kept static or pulsed through the dialyser recipient channel. The resulting dialysate is passed through the TEC, by a second Gilson 401 dilutor. The choice of dialysis mode [static (donor)/static (recipient), static/pulsed or pulsed/pulsed] is described in the Results and Discussion section. After the trace enrichment stage, the injection valve is turned from load to inject and the HPLC mobile phase elutes the retained analytes from the TEC onto the analytical column. The recipient solvent was water and the purging solution for emptying and cleaning of the donor channel was 0.01% (v/v) Triton X-100 in water. The methods for optimising the dialysis (volumes and flow) and the trace enrichment (mass load and volume) are described in the Results and Discussion section.

2.5. Validation parameters and statistics

The parameters that could be influenced by the dialysis system were emphasised in the validation of the method. The specificity of the detection was examined with respect to interference from endogenous plasma components passing through the dialysis membrane, peak separation and baseline resolution in the chromatograms. The iodixanol molecule (Fig. 1) with its multiple rotational isomers [10], gives rise to two characteristic chromatographic peaks called the exo- and endo-isomers, with a ~60:40 area ratio [11], as shown in Results and Discussion (Fig. 5). The linearity of the calibration sample response, as well as the linearity of measurements of control samples against calibration curve, was evaluated by both linear and non-linear regressions of the iodixanol exo- and endo-isomers (for details, see Results and Discussion). Further details concerning determination of limit of detection, limit of quantitation, precision and accuracy are described in Results and Discussion. The robustness of the dialysis and trace enrichment was investigated by additional analyses using the ASTED XL, which is a newer version of the ASTED system.

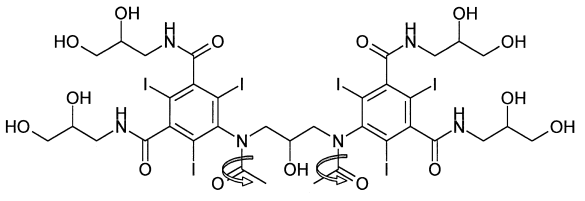

Fig. 1. The molecular structure of iodixanol. The amide bonds, which rotate to give the exo–exo, exo–endo and endo–endo isomers, are indicated by arrows.

3. Results and discussion

3.1. Sample loading capacity

The maximum load of substance on the TEC is dependent on the accessible area of affinity for the analyte and the strength of the affinity to the stationary phase. The first factor is limiting for the maximum amount of substance at optimal binding conditions and the second is important for setting conditions such as flow-rate, volume and solvent strength.

In order to estimate the maximum amount of iodixanol to be retained, a solution of iodixanol in water was continuously injected onto the TEC until the iodixanol breakthrough was detected on the outlet of the column. The flow was 1 ml/min and two concentrations of iodixanol were used for the experiment. A solution of 50 μg/ml could be injected for more than 30 min without any breakthrough. This was equivalent to more than 1.5 mg iodixanol retained. With an iodixanol concentration of 250 μg/ml, a breakthrough of iodixanol could be observed 15 min after start of injection onto the column (Fig. 2), corresponding to 3.75 mg iodixanol as a maximum load for the TEC at this concentration, flow velocity and loading volume.

The maximum enrichment volume for a realistic sample amount and at normal operating conditions was examined by a method described by Andresen et al. [7]. An aliquot of 50 μl of a 1.0 mg/ml solution of iodixanol in water (corresponding to the amount

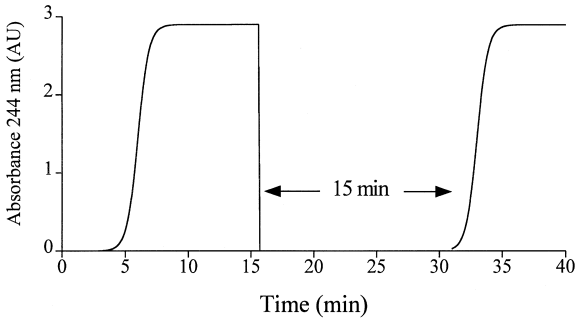

Fig. 2. Trace enrichment column (TEC) breakthrough for a 250 μg/ml solution of iodixanol in water. The column was bypassed by the iodixanol solution until stable detection was obtained (at approx. 16 min.) and subsequently switched in-line with the flow. Breakthrough was observed after 15 min.

of substance in the dialysate from a 1.0 mg/ml control sample after 50% dialysis efficiency) was loaded onto the TEC, which was thereafter flushed with water. At least 30 ml of water could be introduced at a flow of 1 ml/min without any detectable loss of material from the column (Fig. 3). The maximum recipient solution volume necessary for obtaining a satisfying dialysis efficiency is normally a few millilitres, which is far less than the maximum tested TEC loading volume.

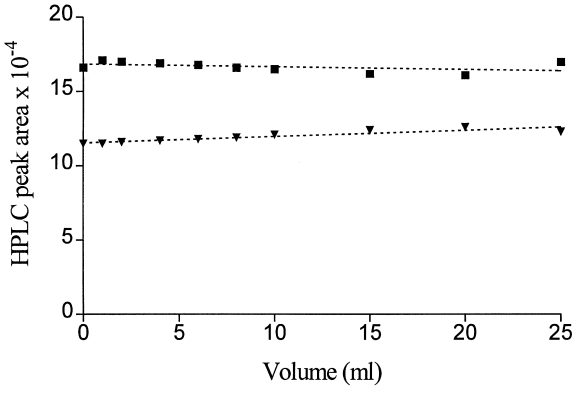

Fig. 3. Trace enrichment column (TEC) retention for 50 μl injection of a 1000 μg/ml solution of iodixanol in water. The column was loaded with the sample, flushed with a volume of water and switched in-line with the mobile phase for quantitative analysis. Volumes of at least 25 ml could be flushed through the column with no loss of the exo-isomer (■) or the endo-isomer (▼).

3.2. Optimisation of the dialysis

A static dialysis recipient solution combined with a static dialysis donor results in a very low dialysis efficiency [12]. A pulsed donor gives a high consumption of sample, and also introduces an additional factor of uncertainty. It has been shown that static donor and pulsed recipient may give up to 50% dialysis efficiency for iopentol [7], which is a monomeric contrast agent structurally related to iodixanol, and this mode was therefore chosen.

The donor volume was optimised to give a fully loaded donor channel, to assure a maximal and reproducible amount of substance. The volume aspirated from the sample vials was adjusted from the default 100 μl to 110 μl, and the total donor volume including the tubing volume was adjusted from the default 180 μl to 185 μl, in order to position the sample perfectly into the donor channel.

A sample of 1 mg/ml iodixanol in water was used for studying the maximum dialysis efficiency over a clean dialysis membrane, i.e., with no proteins or other biological components present. The recipient volume could be increased up to 4 ml before the amount of substance no longer significantly increased (Fig. 4). Further increase up to 5.25 ml resulted in only slightly higher efficiency. The plateau level corresponded to about 55% dialysis efficiency. The dialysis recovery of samples containing undiluted human plasma was about 90% of that of aqueous samples.

3.3. Validation of the results

3.3.1. Specificity

Of the three species, human plasma showed the most complex chromatographic peak pattern of endogenous substances with peaks close to the iodixanol exo- and endo-isomers (Fig. 5). However, near baseline resolution and clear peak detection could be obtained for the iodixanol peaks at all relevant concentrations in human plasma as well as in rat and monkey plasma (not shown).

3.3.2. Linearity

The calibration standards and the control samples were evaluated by linear and non-linear regression analyses. The *y*-axis intercepts of the calibration

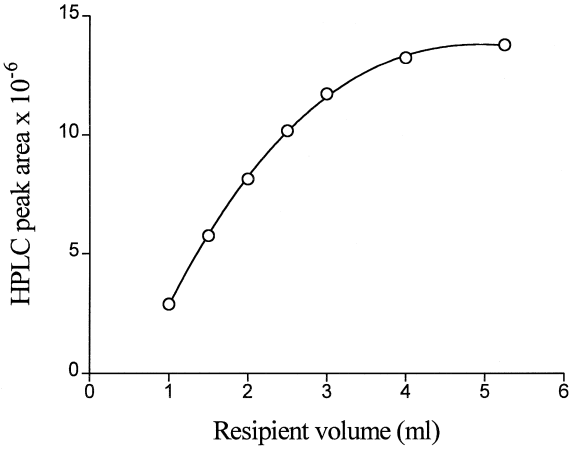

Fig. 4. Dialysis efficiency. A 110-μl volume of a 1000 μg/ml solution of iodixanol in water was kept static in the donor channel while a volume of recipient solution was pulsed through the recipient channel onto the trace enrichment column and thereafter quantitatively analysed. The volume of the recipient solution could be increased up to about 4 ml before the amount of iodixanol in the dialysate no longer significantly increased.

curves, calculated separately for the exo- and endo-isomer HPLC peak areas vs. theoretical concentrations, were equal to zero within the 95% confidence intervals for all individual curves tested (Table 1). These results indicate an absence of significant baseline noise. It also leads to the conclusion that calibration curves should be forced through origo in order to avoid occasional variations, which otherwise would have large consequences for the accuracy at low concentrations. Linear regression analysis of the control samples measured against calibration curve, showed a slope of a value very close to 1 for all species, and with *y*-intercepts not significantly different from zero (Table 2). The results obtained with human, rat and monkey plasma samples were equally good.

No deviation from linearity could visually be shown for the calibration curve, neither by linear nor logarithmic plots (results not shown). Because the concentrations of the samples were ''logarithmically'' distributed, a calculated deviation from linearity over the whole range would have no meaning if determined from the linear plotted values; i.e., the low values would have minor influence on the results even if their relative deviation from linearity (relative residuals) were large. The linearity factors (*m*) were therefore determined for the log-transformed plots. The determined *m*-values for the exo- and endo-iodixanol calibration curves and the control samples for the three species all satisfied the suggested criterion for linearity ($0.90<m<1.10$) [13], and the correlation factors (*r*) were all better than 0.9990 (Table 3).

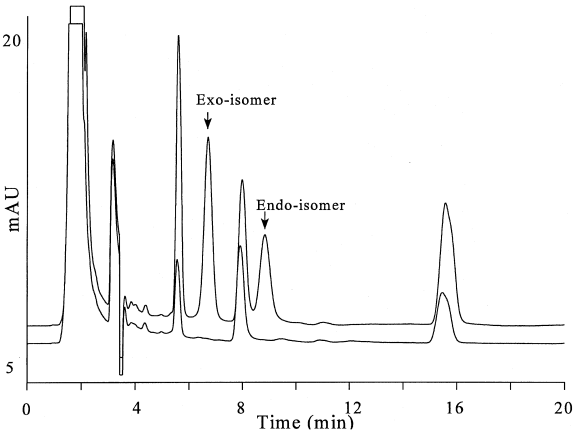

Fig. 5. Chromatogram of normal human plasma (lower trace) and normal human plasma spiked with 5.0 μg iodixanol/ml (upper trace). The characteristic exo- and endo-isomer peaks of iodixanol are indicated. All other peaks are endogenous components of the plasma.

3.3.3. Precision and accuracy

Series of control samples with plasma of the three species, in the range 0.5–500.0 μg/ml, were repeatedly analysed six times for 6 or more days and results were calculated from standard curves made from human plasma samples. The inter- (between-) assay precision varied from 1.3 to 13.0% RSD (Table 4). The lowest concentration of 0.5 μg/ml is close to the limit of quantitation (LOQ) values (see Section 3.3.4) and therefore has a lower precision than that obtained at higher concentrations. The intra- (within-) assay accuracies were better than the inter-assay accuracies for all species at all concentrations (not shown).

The inter- (between-) assay accuracies relative to the human plasma standard curves were slightly above 100% for human and monkey samples and about 90% for rat samples (Table 4). The differences between the human, rat and monkey samples may

Table 1
Validation of iodixanol exo- and endo-isomer calibration curves, all in human plasma, with respect to the *y*-axis intercepts after linear regression[a]

Iodixanol-isomer	Analytical series no.	Intercept±SE		95% CI	
		Lowest	Highest	Lowest	Highest
Exo	I	12.2±13.4	17.1±19.0	−25↔49	−36↔70
	II	−5.7±3.3	5.1±7.1	−16↔5	−18↔28
	III	−1.1±3.1	8.8±12.1	−10↔8	−25↔42
Endo	I	−2.6±1.6	6.2±8.0	−7↔2	−16↔28
	II	−3.3±1.5	1.4±2.9	−8↔2	−8↔11
	III	−4.3±2.2	3.6±6.2	−10↔2	−14↔21

[a] The lowest and highest values obtained after analysis of seven or more individual curves are presented for three analytical series, with standard errors (SEs) and the corresponding calculated 95% confidence intervals (CIs).

Table 2
The mean values of the slopes and of the *y*-axis intercepts, after linear regression analysis of six or more analytical series of control samples, with standard errors (SEs) and the corresponding calculated mean 95% confidence intervals (CIs) for the intercepts

Species	Slope±SE	Intercept±SE	95% CI
Human	1.058±0.000	0.086±0.28	−0.4↔0.6
Rat	0.947±0.002	−0.60±0.44	−2.5↔1.3
Monkey	1.043±0.001	−0.35±0.28	−1.5↔0.9

reflect different analysis recoveries from the different animal plasmas, similar to the 10% difference in recovery observed for iodixanol in water vs. iodixanol in human plasma (see Section 3.2). Although all the values are within the normally accepted limit of ±15%, such species dependent deviations should be carefully inspected. However, the method is fully valid if a proper set of control samples is included

Table 3
Linearity factors (*m*) from non-linear regression analysis and correlation coefficients (*r*) from linear regression analysis of the exo- and endo-iodixanol calibration curves and the control samples[a]

	Human		Rat		Monkey	
	m	*r*	*m*	*r*	*m*	*r*
Exo-iodixanol	1.08	0.9993	1.01	0.9999	0.98	0.9998
Endo-iodixanol	0.98	0.9999	0.98	0.9999	0.09	0.9998
Control samples	1.02	1.0000	1.02	0.9997	1.01	1.0000

[a] All values are the result of six or more experiments, and with logarithmic transformation of the curves before the regression analyses.

Table 4
Inter-assay precision and accuracy of analysis of human ($n=5$), rat ($n=7$) and monkey ($n=5$) plasma control samples, expressed as relative standard deviation (RSD) and % of theoretical concentration (%±SD), respectively

Control sample (μg/ml)	Precision (RSD, %)			Accuracy (%±SD)		
	Human	Rat	Monkey	Human	Rat	Monkey
0.5	5.7	8.6	13.0	110±6.3	90±7.7	101±13
5.0	1.3	3.3	2.8	105±1.4	86±2.8	101±2.8
50.0	1.3	2.6	3.9	106±1.4	92±2.4	103±4.0
500.0	1.4	2.2	2.7	106±1.5	95±2.1	104±2.8

and the results of these are used for correcting the results of the sample analyses.

3.3.4. Limit of detection and limit of quantitation

The limit of detection (LOD) was estimated as $LOD = mean_{bl} + 3SD_{bl}$, where $mean_{bl}$ is the mean value of several measurements of blank plasma sample and SD_{bl} is the corresponding standard deviation. The LOQ was similarly estimated as $LOQ = mean_{bl} + 10SD_{bl}$. The values obtained for LOD and LOQ for human, rat and monkey plasma are shown in Table 5. One problem with blank plasma measurements is the occasional lack of detectable HPLC peaks and hence no concentration to be determined. The result of this is unrealistic low LOD and LOQ values. Therefore, in Table 5, next to the values which include one or more zero concentration samples, the results of sets of blank samples with only positively detected peaks are given. Using the above definition, the determined LOQs were relatively low. As the precision and accuracy were not determined in that range of concentration, it was suggested that the LOQ was defined to be the concentration of the lowest documented control sample: 0.5 μg/ml. An ''upper limit of quantitation'' was determined by a combination of detector range, linearity of measurements, precision and accuracy. Preliminary experiments showed that the detector limit, which was electronically set to 2.0 AUFS, limited the maximum sample concentration to be somewhere between 500 and 1000 μg/ml. The upper limit was therefore defined as the highest control sample concentration which satisfied the requirements for linearity, precision and accuracy, i.e., 500 μg/ml.

Table 5
Limit of detection (LOD) and limit of quantitation (LOQ) determined from analyses of human, rat and monkey blank plasma samples

Species	LOD (μg/ml)	LOQ (μg/ml)
Human ($n=33^a/n=19^b$)	$0.110^a/0.13^b$	$0.310^a/0.34^b$
Rat ($n=7$)	$0.019^a/0.20^c$	0.052^a
Monkey ($n=12$)	$0.035^a/0.20^c$	0.096^a

[a] All measurements, also including zero values from not-detected noise-peaks.

[b] Including only positively detected noise-peaks.

[c] Using the mean values from blank samples and the SD values from control samples 0.5 μg/ml (due to almost only zero blank values).

3.3.5. Analyte stability

Iodixanol (as non-ionic X-ray contrast agents in general) is an extremely stable substance. An intravenous injection of iodixanol was excreted in the urine with recoveries up to 99% over a period of 3 days, without showing any indications on biotransformation or degradation [8]. We have shown that iodixanol is stable in human plasma for at least 16 h at room temperature, for at least 4 weeks at 2–8°C and for at least 24 weeks at −20°C, and is unaffected by three freeze–thaw cycles. Similar results were obtained for iodixanol in rat and monkey plasma.

3.3.6. Robustness

Several series of calibration samples and control samples were analysed on a second HPLC system equipped with ASTED XL, the newer version of the on-line dialysis system. The ASTED XL differs from the ASTED system in that the tubings have other dimensions, the ASTED XL has two Rheodyne valves in function and the programming is different in that it is built up of predefined steps (''tasks'') in contrast to the more freely programmable ASTED. Only samples with human plasma were analysed and compared. The chromatograms (not shown) were almost identical to the ones of the ASTED HPLC system; i.e., the specificity was not altered by use of ASTED XL. The linearity, tested by linear and non-linear regression analysis, and the precision were equally satisfying with the ASTED XL system as the ASTED system (Table 6). The precision of the 0.5 μg/ml control sample indicates that LOQ of the ASTED XL system is similar to that of the ASTED system.

4. Conclusion

A fully automated method for analysing iodixanol in plasma has been developed. The method is selective for the substance, sensitive down to 84 pmol (0.13 μg)/ml and has a high precision over linear range up to 320 nmol (500 μg)/ml. The online dialysis and trace enrichment process is robust in that

Table 6
Comparison of the performances of the HPLC systems equipped with ASTED and ASTED XL[a]

Parameter	Sample	ASTED XL	ASTED
95% confidence interval	Calibration exo-isomer	−63↔133	−25↔49
	Calibration endo-isomer	−11↔20	−7↔2
	Control samples	−0.0015↔0.0024	−0.4↔0.6
Linearity factor (*m*)	Calibration exo-isomer	1.02	1.08
	Calibration endo-isomer	1.03	0.98
	Control samples	1.04	1.02
Correlation factor (*r*)	Calibration exo-isomer	0.9997	0.9993
	Calibration endo-isomer	0.9995	0.9999
	Control samples	0.9999	1.0000
Precision (intra-assay)	Control sample 0.5 μg/ml	5.0%	5.8%

[a] All values are the result of six or more experiments.

no differences in the results from systems with ASTED and with the newer version ASTED XL could be found. The method has been used in the determination of pharmacokinetic parameters in a clinical phase I study [14]. Although developed for iodixanol, the method can easily be modified and applied to other substances with similar properties, i.e., substances having low protein binding and high water solubility but strong enough stationary phase affinity to be retained by an appropriate chosen trace enrichment column.

Acknowledgements

I want to thank Heidi Meyer-Mørch, Mari Tøftum and Grete A. Friisk for outstanding laboratory work and Tore Skotland for valuable discussions and comments.

References

[1] D.C. Turnell, J.D.H. Cooper, J. Chromatogr. 395 (1987) 613.
[2] J.D.H. Cooper, D.C. Turnell, B. Green, F. Verillon, J. Chromatogr. 456 (1988) 53.
[3] M. Krogh, A.S. Christophersen, K.E. Rasmussen, J. Chromatogr. 621 (1993) 41.
[4] R. Herraez, A.J. Louter, N.C. van de Merbel, U.A. Brinkman, J. Pharm. Biomed. Anal. 14 (1996) 1077.
[5] J.R. Veraart, J. van Hekezen, M.C. Groot, C. Gooijer, H. Lingeman, N.H. Velthorst, U.A. Brinkman, Electrophoresis 19 (1998) 2944.
[6] E. van Bakergem, R.A. van der Hoeven, W.M. Niessen, U.R. Tjader, J. van der Greef, G.K. Poon, R. McCague, J. Chromatogr. 15 (1992) 189.
[7] A.T. Andresen, P.B. Jacobsen, K.E. Rasmussen, J. Chromatogr. 575 (1992) 93.
[8] P.B. Jacobsen, L. Blindheim, T. Skotland, Acta Radiol. 36 (Suppl. 399) (1995) 61.
[9] P.B. Jacobsen, Am. J. Neuroradiol. 13 (1992) 1521.
[10] R. Fossheim, T. Guldbrandsen, H. Priebe, A.J. Aasen, Acta Chem. Scand. 49 (1995) 589.
[11] H. Priebe, H. Dugstad, M. Gacek, E. Hagen, O.M. Homestad, Å. Larsen, C.E. Sjøgren, T. Thomassen, Acta Radiol. Suppl. 399 (1995) 21.
[12] D.C. Turnell, J.D.H. Cooper, J. Autom. Chem. 7 (1985) 177.
[13] E. Debesis, J.P. Boehlert, T.E. Givand, J.C. Sheridan, Pharm. Technol. September (1982) 120.
[14] P. Höglund, P. Leander, S.O. Hustvedt, Y. Kloster, A. Børseth, Acad. Radiol. 5 (Suppl. 1) (1998) S47.