Column-switching HPLC–MS/MS analysis of ropivacaine in serum, ultrafiltrate and drainage blood for validating the safety of blood reinfusion

Torben Breindahl [a],[*], Ole Simonsen [b], Kirsten Andreasen [a]

[a] Department of Clinical Biochemistry, Vendsyssel Hospital, Bispensgade 37, DK-9800 Hjørring, Denmark
[b] Orthopaedic Clinic (Part of Orthopaedic Division, North Denmark Region), Vendsyssel Hospital, Barfredsvej 8, DK-9900 Frederikshavn, Denmark

ARTICLE INFO

ABSTRACT

A high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS–MS) method, using back-flush column-switching was developed for total drug concentrations of ropivacaine in serum and drainage blood in the measuring range 0.1–10 μg/mL. Samples were diluted with internal standard (^{2}H$_7$-ropivacaine) and extraction buffer, centrifuged and injected directly onto a BioTrap 500 MS extraction column. Using a time programmed six-port valve switch, ropivacaine was back-flushed onto a Zorbax SB-Aq analytical column, gradient eluted and finally detected after electro spray ionisation and multiple reaction monitoring (MRM) of the transitions m/z 275 → m/z 126 and m/z 282 → m/z 133 for ropivacaine and ^{2}H$_7$-ropivacaine, respectively. Accuracy (bias-%) was −1.5 to 5.8% and intermediate precision (C.V.) was 1.4–3.1%. The low sample amount required (10 μL), high specificity and short run time (6 min) makes it very suitable for determination of ropivacaine. Using the same methodology as described above and 200 μL ultrafiltrate, the free drug concentrations of ropivacaine in serum could be precisely determined with a C.V. below 3%. The method was used to investigate the safety of reinfusion of drainage blood after knee and hip arthroplasty when ropivacaine (Naropin®) was used for local analgesia. Data for 30 patients are summarised.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Ropivacaine, (S)-1-propyl-2′,6′-pipecoloxylidide (Naropin®, AstraZeneca), is a long-acting amide-type local anaesthetic which is used in high concentrations for surgical anaesthesia and lower concentrations for post-operative pain relief. Local infiltration analgesia (LIA) using ropivacaine is generally recommended in knee and hip arthroplasty. Ropivacaine can be used in larger amounts than the analogue drug bupivacaine because of its lower cardiotoxicity in such major orthopaedic procedures. Due to blood loss, blood transfusion is often required. Blood reinfusion is generally highly recommended compared to allogenic transfusion which involves numerous potential risk factors. However, in patients receiving LIA, reinfusion of drainage blood implies risk of central nervous system (CNS) or cardiovascular system (CVS) toxicity due to potentially toxic concentrations of ropivacaine. By researching this subject, Stringer et al. showed data that strongly indicated the safety of reinfusion [1] and recently Parker et al. published the first promising results from a study among a few otherwise healthy patients receiving reinfusion after knee arthroplasty [2]. However, knee and hip arthroplasty is often performed among elderly and

patients with significant comorbidity and the safety of routine blood reinfusion involving such patients has hitherto not been studied. Also the concentration of ropivacaine in drainage blood from larger consecutive groups of patients and various injection techniques to minimize the concentration has not been studied. If safe, routine combination of LIA and blood reinfusion will be a major step forward in knee and hip surgery to reduce the risk of complications, decrease length of hospitalisation and save highly costly donor blood for other purposes. Before implementation, hospitals worldwide will be expected to confirm and fully validate the safety of blood reinfusion according to local quality procedures. Consequently, a precise analytical technique for determination of the relevant anaesthetics is highly needed.

Analytical methods for ropivacaine in biosamples have mainly been based upon sample preparation by liquid/liquid extraction [1–4], protein precipitation (PPT) [5,6], solid-phase extraction (SPE) [7], solid-phase micro-extraction (SPME) [8] or molecular imprint-based solid-phase extraction (MISPE) [9]. Separation was done by gas chromatography with either nitrogen-phosphorous detection (GC–NPD) or mass spectrometric detection (GC–MS) [8,10–12] or by high-performance liquid chromatography with either ultra violet detection (HPLC-UV) [1–4,] or mass spectrometric detection (HPLC–MS, HPLC–MS/MS) [5–7,9,13]. The drawbacks of some of these methods are high sample volumes and elaborate, time-consuming clean-up techniques that are difficult to automate.

* Corresponding author. Tel.: +45 99644103; fax: +45 99646232.
E-mail address: torben.breindahl@rn.dk (T. Breindahl).

Several methods were dedicated to the study of free (unbound) concentrations after ultrafiltration [14] or microdialysis [6,7,13]. A few methods included quantification of metabolites for pharmacokinetic studies [5,12]. Some early publications described on-line extraction by column-switching techniques for anaesthetics in serum by HPLC-UV techniques [14–18]. Although these methods performed excellent they lacked the high specificity that was later provided when routine HPLC–MS/MS systems became available. A new and very promising technique is the use of micro-extraction in packed syringes (MEPS) coupled to HPLC–MS/MS for ropivacaine and metabolites in serum [19–21].

The present study was specifically aimed at developing a fast, precise, accurate and highly automated analytical approach for the quantification of ropivacaine in both serum and drainage blood in order to support local research activities into the safety and application of post-operative reinfusion. This is to the authors' best knowledge the first on-line column-switching HPLC–MS/MS method validated and used for analysis of ropivacaine in drainage blood. The method was used in a pilot study involving 30 patients undergoing knee or hip arthroplasty. Method validation data and a summary of patient sample results are presented here.

2. Experimental

2.1. Materials and reagents

Organic solvents and reagents were of analytical grade. Acetonitrile was isocratic (HPLC) grade from Merck. Purified water (18.2 MΩ) was prepared on an Elga Centra RDS system (Buckinghamshire, UK). A reference standard of S-($-$)-1-propyl-2′-6′-pipecoloxylidide hydrochloride monohydrate (ropivacaine) was kindly provided by AstraZeneca UK Ltd. The internal standard ^{2}H$_7$-ropivacaine was from C/D/N Isotopes Inc., Quebec (Canada).

2.2. Mobile phases

Mobile phase A consisted of 4% 2-propanol in 10 mM ammonium acetate adjusted to pH 6.5 with concentrated formic acid. Mobile phase B was acetonitrile. The mobile phase for extraction was 4% 2-propanol in 10 mM ammonium acetate adjusted to pH 10 with 25% (w/w) ammonium hydroxide solution.

2.3. Standard solutions

Stock solutions (1 mg/mL) with concentrations corresponding to the free bases of ropivacaine and internal standard (^{2}H$_7$-ropivacaine) were prepared in purified water and stored at +4 °C.

2.4. Calibrators and quality control samples

Serum calibrators (n = 10) were prepared by spiking appropriate volumes of aqueous working solutions of ropivacaine into serum. The concentrations of ropivacaine were 0.1, 0.25, 0.5, 1, 2.5, 5, 6, 7, 9 and 10 μg/mL. A 10-point calibration curve was constructed for each batch of samples, with serum calibrators, serum control samples and patient samples undergoing similar sample pre-treatment procedures (Section 2.6.1). Standard curves based on peak area with a quadratic curve fit ($Y = aX^2 + bX + c$), not forced through zero, with weighting (1/x) was used for calibration.

Quality control samples in three levels used for validation were prepared by spiking appropriate volumes of aqueous working solutions into serum or drainage serum. The concentrations of ropivacaine were 0.25, 5 and 9 μg/mL. Blank pooled serum and drainage serum samples used for spiking were free of ropivacaine and centrifuged at 3000 × g for 10 min before use. All calibrators and quality control samples were aliquoted and kept frozen (below -18 °C) until used.

2.5. Projects samples

Blood samples and drainage blood were collected from 30 patients from Orthopaedic Clinic (Vendsyssel Hospital, Frederikshavn, Denmark) undergoing either knee arthroplasty (n = 20) or hip arthroplasty (n = 10) including local infiltration analgesia (LIA) with ropivacaine (Naropin®), 3 mg per kg body weight (max. 200 mg). Before closure of the wound the volume was infiltrated by needle into the joint capsule, muscles and subcutaneous tissue around the wound, and a 12 gauge drain and a 20 gauge (epidural) catheter was introduced into the joint cavity. This catheter was used for administration of bolus injection of 150 mg ropivacaine (20 mL Naropin®, 7.5 mg/mL) at 22 p.m. on the day of surgery and in the following morning at 7 a.m. Venous blood was sampled at 2 and 6 h after closure of the wound. Blood drained during the first 6 h was collected for analysis. The total drain volumes were measured, the fluids were centrifuged at 3000 × g for 10 min and aliquots of drainage blood serum (hereafter referred to as "drainage serum") were isolated. All samples were kept frozen (below -18 °C) until analysis.

2.6. Sample preparation

2.6.1. Total concentrations of ropivacaine in serum and drainage serum

Sample preparation was equivalent for calibrators, quality controls and patient samples. Serum or drainage serum (10 μL) was mixed with 10 μL aqueous working solution of ^{2}H$_7$-ropivacaine (2 μg/mL) and 1000 μL 4% 2-propanol in 10 mM ammonium acetate (pH 10) in a standard 2 mL glass vial. After capping the vial was shaken, centrifuged at 3000 × g for 5 min and placed in the autosampler. A Gilson Microman (M10) capillary-piston pipette was used for the small volumes. Samples were diluted and reanalysed if results were above 10 μg/mL.

2.6.2. Free concentrations of ropivacaine in serum

Serum (500 μL) was transferred onto a Microcon Ultracel YM-30 ultrafiltration device (Millipore). The tube was centrifuged for 30 min at 12,000 × g. Then 200 μL of the resulting ultrafiltrate was transferred into a standard 2 mL glass vial containing 10 μL aqueous working solution of ^{2}H$_7$-ropivacaine (2 μg/mL) and 810 μL 4% 2-propanol in 10 mM ammonium acetate (pH 10). After capping the vial was shaken, centrifuged at 3000 × g for 5 min and placed in the autosampler. A multiplication factor of 0.05 was used during calculation of the final results.

2.7. Liquid chromatography

The HPLC system modules were all from Agilent Technologies (Palo Alto, CA, USA) including a 1200 binary pump, 1200 SL autosampler and 1200 column department with a six-port switching valve embedded. The damper and mixer were bypassed in order to optimize the pumping system to low dead-volume as described in the Agilent User Manual. Autosampler injection volume was 10 μL. The analytical column was an Agilent Zorbax SB-Aq, 50 mm × 2.1 mm, i.d., packed with 3.5 μm particles. Flow rate was 600 μL/min. Column temperature was 40 °C. The binary pump gradient started at 20% phase B for 1.5 min and then went up to 90% phase B from 1.5 to 4 min. It was maintained at 90% phase B for 0.5 min and then brought back to initial conditions for 1.5 min of equilibration. Total run time was 6 min. The 6-port switching valve brought the BioTrap extraction column in series with the analytical column for back-flush elution after 1.5 min and was

switched back at 4.5 min thus allowing for 1.5 min equilibration before next sample injection. Extraction was controlled using an Agilent 1100 isocratic pump directly connected to the autosampler. The extraction column was a BioTrap 500 MS, 20 mm × 2.0 mm, i.d. (Chromtech, Sweden). Flow rate was 1 mL/min during extraction and programmed to 0.05 mL/min when pumping directly to waste. The BioTrap effluent going to waste during extraction was monitored with an Agilent 1100 diode-array detector (DAD) at wavelengths 190–400 nm.

2.8. Tandem mass spectrometry

2.8.1. Mass spectrometric conditions

The MS system consisted of a Sciex QTRAP 3200 mass spectrometer (Applied Biosystems, CA, USA) equipped with a TurboIon spray source operated in positive mode. A time programmed switching valve directed the eluent to the detector between 0.5 and 5.5 min, otherwise to waste. The eluent was split 1:1 using 0.12 mm i.d. polyetheretherketone (PEEK) tubing in a low dead-volume tee at the spray source. Ion spray voltage was 5500 V, source temperature 550 °C, ion gas 1 and 2 were 50 p.s.i., curtain gas was 22 p.s.i. and depolarisation potential 58 V. Multiple reaction monitoring (MRM) parameters for ropivacaine were: $m/z\ 275 \rightarrow m/z\ 126$ (collision energy: 35, signal used for calibration), $m/z\ 275 \rightarrow m/z\ 84$ (collision energy: 73, first qualifying ion), $m/z\ 275 \rightarrow m/z\ 56$ (collision energy: 75, second qualifying ion). For internal standard $^2H_7$-ropivacaine: $m/z\ 282 \rightarrow m/z\ 133\ m/z$ (collision energy: 31, signal used for calibration), $m/z\ 282 \rightarrow m/z\ 105$ (collision energy: 53, first qualifying ion), $m/z\ 282 \rightarrow m/z\ 85$ (collision energy: 55, second qualifying ion). Dwell time was 150 ms at unit resolution.

2.8.2. Evaluation of matrix effects

Matrix effects caused by potential interfering substances (endogenous matrix compounds) was monitored by injection of randomly selected serum samples ($n = 6$) from the Department of Clinical Biochemistry (Vendsyssel Hospital, Denmark). Ion suppression was monitored using post-column syringe pump infusion of 10 µg/mL ropivacaine solution in mobile phase A (flow 25 µL/min) via a low dead-volume cross to connect the four PEEK tubes (column, split, ionisation source and syringe pump). After equilibration blank drug-free serum was injected and the change in baseline near the retention time of ropivacaine was monitored. Additionally, the relative MS-detector response was calculated for serum calibrators versus aqueous solutions of ropivacaine injected into the column-switching system in equal concentrations.

2.9. Method validation

2.9.1. Total concentrations of ropivacaine in serum and drainage serum

Intra-assay precision and accuracy were assessed by replicate analysis of quality control samples ($n = 5$) at three levels (0.25, 5 and 9 µg/mL). Precision was reported as coefficient of variation (C.V.%) and accuracy (bias-%) expressed as [100 − (mean calculated concentration/spiked concentration) × 100%] equal to [recovery (%) − 100]. Intermediate precision was assessed by analysis of quality control samples at three levels (0.25, 5 and 9 µg/mL) in different batch runs ($n = 5$). Furthermore, one authentic patient serum and one drainage serum sample was analysed in replicates ($n = 5$) during the validation.

2.9.2. Free concentrations of ropivacaine in serum

Free concentrations were determined in 14 serum samples from hip arthroplasty patients ($n = 7$). Intra-assay precision was assessed by repetitive analysis of a patient serum sample ($n = 5$) and reported

as coefficient of variation (C.V.%). Intermediate precision and accuracy was not studied as the equilibrium of free and protein-bound ropivacaine in serum is due to change during storage [6]. To test if ultrafiltrates could be frozen and later analysed in the same batch, ultrafiltrates from patient samples ($n = 18$) were kept frozen below −18 °C for 4 weeks and reanalysed afterwards.

2.9.3. Method comparison

Frozen serum containing ropivacaine ($n = 25$) were kindly provided by AstraZeneca R&D (Södertälje, Sweden). Some samples also contained bupivacaine ($n = 11$). They were analysed and results compared with the reference values. The analytical method used at the Department of Clinical Pharmacology & DMPK (AstraZeneca R&D, Södertälje, Sweden) for determination of total concentrations of ropivacaine in plasma is based on ultrafiltration (after minimising protein binding) followed by reversed-phase liquid chromatography with tandem mass spectrometric detection using $^2H_7$-ropivacaine as internal standard.

3. Results and discussion

The direct extraction of protein-containing matrices with coupled-column chromatography or two-dimensional LC-system in back-flush mode is well known [22,23]. Using the BioTrap as precolumn for the extraction of polar drugs from plasma or serum has been thoroughly investigated [24,25]. The BioTrap 500 MS column particles are manufactured with a coating of α1-acid glycoprotein (AGP) on the external surface and consequently compatible with various types of protein-containing biosamples under generic conditions for either basic or acidic compounds. Similar to other restricted access media (RAM) extraction columns the small, polar molecules are retained after diffusing into the inner hydrophobic surface, while macromolecules are excluded and flushed to waste. Extraction time, flow, pH, buffer and content of organic solvent are parameters for optimisation, but small fluctuations from the settings presented here do not compromise performance of the method (data not shown). The BioTrap column can tolerate high injection volumes of serum without deterioration and consequently the column was never changed throughout this study. By monitoring the effluent passing to waste during extraction by a DAD it could be verified that a steady baseline signal was obtained
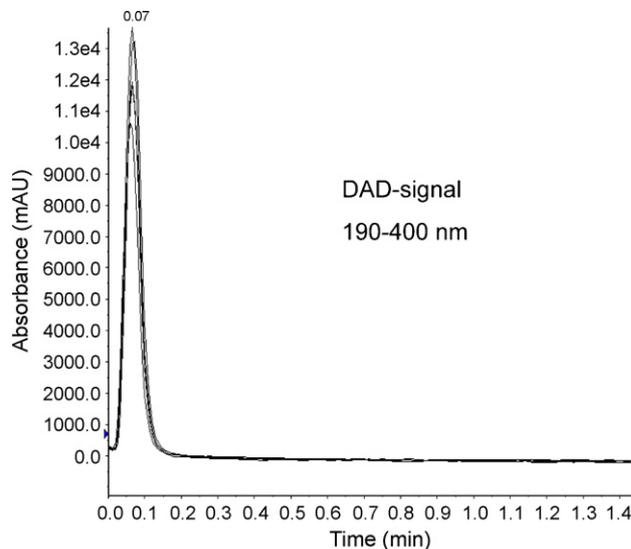
Fig. 1. Diode-array-detector (DAD) signal (190–400 nm) monitoring the effluent from the BioTrap 500 MS extraction column. Y-axis: absorbance (0–13,000 mAU); X-axis: time 0–1.45 min. Signals from 5 different patient serum samples are overlaid. Plots for drainage serum are equivalent.
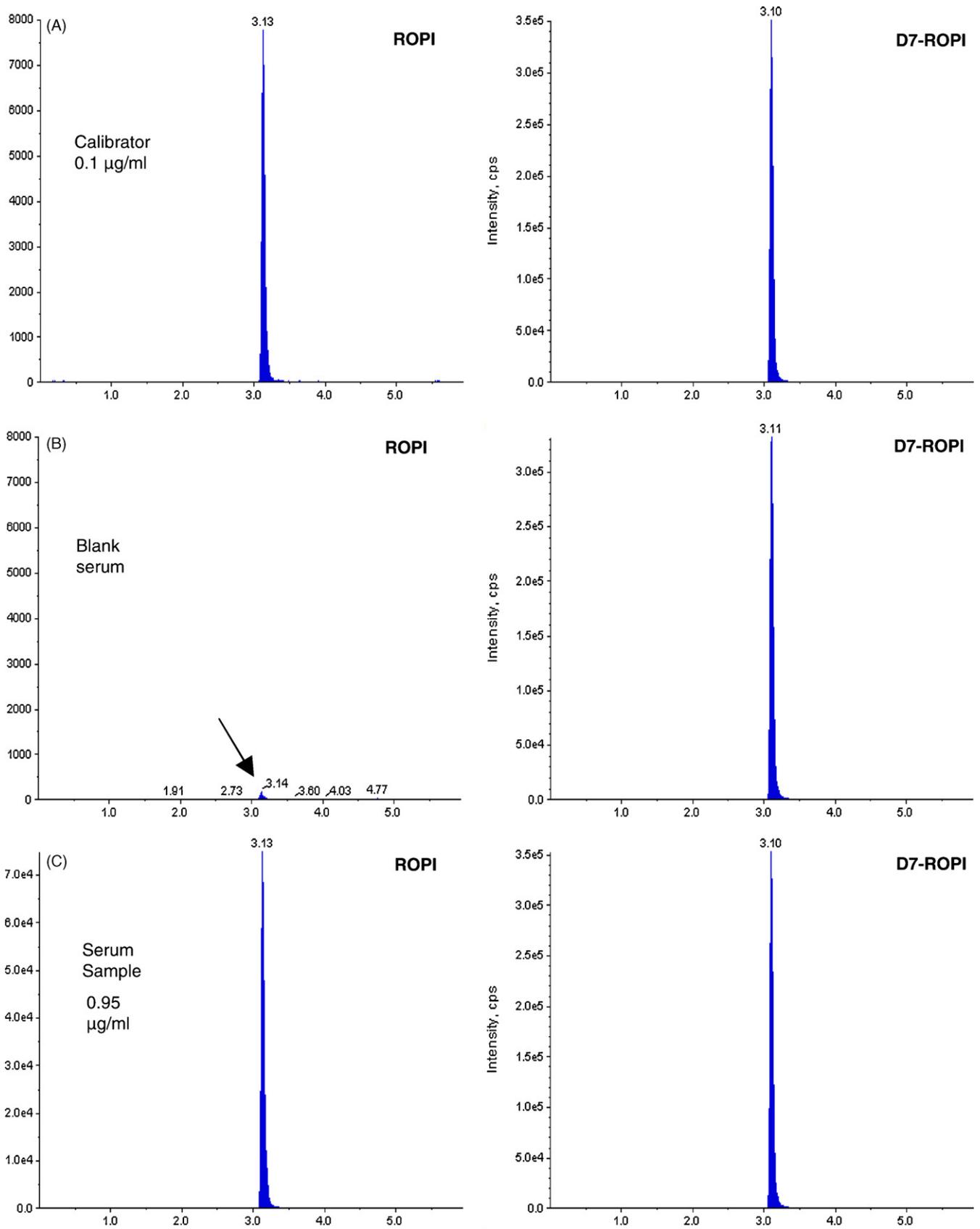
Fig. 2. Multiple reaction monitoring (MRM) chromatograms of ropivacaine: $m/z\ 275 \rightarrow m/z\ 126$ (left columns) and $^2H_7$-ropivacaine: $m/z\ 282 \rightarrow m/z\ 133$ (right columns) for (A) lowest serum calibrator 0.1 μg/mL; (B) blank serum; (C) patient serum sample containing ropivacaine (0.95 μg/mL). Chromatograms A and B for ropivacaine (left columns) are in the same scale. Blank signal indicated by arrow.

Table 1
Method validation data ($n = 5$).

Sample matrix	Concentration (μg/mL)	Intra-assay precision		Intermediate precision	
		C.V. (%)	Bias (%)	C.V. (%)	Bias (%)
Spiked serum	0.25	1.0	−1.0	3.1	+0.2
	5.0	1.2	+1.3	2.0	+0.8
	9.0	1.6	+1.1	2.4	+1.4
Spiked drainage blood	0.25	1.1	−2.9	1.6	−1.5
	5.0	2.9	+3.1	1.4	+2.1
	9.0	1.2	+6.0	2.2	+5.8
Patient serum	0.29	1.5			
Patient drainage blood	3.2	0.7			
Patient ultrafiltrate	0.056	2.8			

before "back-flush" elution was initiated (Fig. 1). It was concluded that the extraction time of 1.5 min was sufficient because the major UV-signals from proteins and other matrix compounds eluted early and a steady baseline was obtained already after 1 min.

Calibration curves (0.1–10 μg/mL) were reproducible and showed acceptable curve fit with correlation coefficients (r^2) above 0.99. A quadratic calibration curve fit has previously been used in HPLC–MS/MS analysis of ropivacaine [5,6,19,20]. Chromatograms were free of interferences for all calibrators and samples making auto integration and data handling very easy (Fig. 2). Because the compounds were eluted back and forward between two columns, the peak shapes were not highly symmetrical. Nevertheless, retention times were very reproducible with intra-assay variations less than 0.4%. Autosampler carry-over was acceptable, the worst case observed was 0.06% peak area from the highest calibrator (10 μg/mL) to blank, thus with minimal effect on the assay.

Matrix effects are quite common for biomedical analysis with HPLC–MS [26], and this method was no exception, however it had no critical effect on the quantification process. The response of serum calibrators relative to aqueous working solutions of equal concentration was about 92%. The infusion experiment showed ion suppression and ion enhancement peaks that would interfere with early eluting compounds, but there was less than 10% baseline drift in the region around the retention time of ropivacaine. However, it is generally accepted that a deuterium-labelled internal standard (in this case: ^{2}H$_7$-ropivacaine) that coelutes with the target compound (ropivacaine) compensates for the alteration in signal within the HPLC gradient run and for temporary fluctuations in sensitivity within or between batches, thereby minimising matrix and ion suppression effects on the quantification results [26].

Method validation data (Table 1) showed excellent intra-assay precision and accuracy for all concentrations in both matrices. Accuracy showed bias-% up to 6% for spiked drainage serum at high concentrations. The method comparison with AstraZeneca with 25 serum samples was carried out due to the absence of other external quality materials as ropivacaine is not routinely monitored at clinical biochemistry laboratories. The data indicated excellent agreement between the two methods showing a correlation coefficient (r^2) of 0.998, linear regression line slope of 1.0086 and y-axis intercept of −0.0185. These validation data were considered fully adequate for the purpose of the method and well in line with international acceptance criteria for biomedical analysis. By comparing the present column-switching technique with other off-line clean-up and sample pre-treatment methods coupled to tandem mass spectrometry, the authors find that an automated removal of proteins using column-switching is a superior approach with better perspectives for further improvement. It is generally accepted that PPT in acetonitrile, although widely used, does not result in a very clean extract and will be a potential cause for significant ion suppression as stated in a recent review on matrix interference [26]. On the contrary, advanced use of column-switching can result in

multi-dimensional on-line extraction by coupling several extractions columns based on size exclusion, mixed-mode ion exchange, molecularly imprint polymers (MIPs) or other RAM materials. The end result is a more robust and precise method which can be observed in the low C.V.s for the present method compared to other mass spectrometric methods for ropivacaine [5–7]. Additionally, when working with column-switching the injection volume is not a limiting factor or parameter, which in the case of PTT can cause distorted peak shapes due to the solvent effect of acetonitrile.

Mass spectrometric data acquisition included two qualifying ions for both ropivacaine and ^{2}H$_7$-ropivacaine. These data are generally used for identification purposes, but in this case they were included to be able to monitor matrix effects and interferences, e.g. for outlying internal standard response within the batch run. The relative ion intensities of qualifying ions in samples, expressed as the percentage of the intensity of the most intense MRM transition for each compound, showed a variation less than ±20% compared with averaged values for calibrators in the same batch. This complies with the narrowest tolerance criteria commonly used for LC–MS, hence it was concluded that no matrix interference could be recognised by this approach. Additionally, internal standard response showed no trend of loss in sensitivity within the batches.

Signal-to-noise ratio (S/N) on the lowest calibrator (0.1 μg/mL) was typically 190 and the estimated limit-of-detection (LOD) for the MRM transition m/z $275 \rightarrow m/z$ 126 was 0.002 μg/mL. No patient samples in this study had total ropivacaine concentrations below the lowest calibrator (0.1 μg/mL), which was chosen as the lower limit of quantification (LLOQ). High sensitivity is not a key parameter in this study as low ng/mL plasma levels are well tolerated by the patients and therefore irrelevant to measure with high precision. Other ropivacaine assays are normally calibrated at lower levels, but here a measurement range up to 10 μg/mL was chosen to include both the cardiovascular toxicity range for total ropivacaine in plasma (about 6–10 μg/mL) and the relative high drainage serum levels expected during the study. The upper limit of quantification (ULOQ) was defined by the highest standard. All biosamples could be analysed using this same calibration curve, with the exception of seven drainage serum samples that were diluted (1:1) and reanalysed. Other investigations have documented the stability of ropivacaine in serum for months at −20 °C [3] and in spiked serum samples at room temperature for at least 48 h [6] hence the absence of a study in this paper.

Ropivacaine is extensively bound to plasma proteins (mainly to α1-acid glycoprotein) with the free fraction around 6–10% inducing toxicity above 0.6 μg/mL [27]. One of the best analytical tools to assess the risk of CNS or CVS toxicity caused by local anaesthetics during reinfusion is determination of the free concentration of the drug in serum. The availability of α1-acid glycoprotein as a binding partner is a key factor with large inter- and intra-individual variability and consequently different binding capacities. This protein increases as a response to inflammation after surgery and

Table 2
Project summary: Determination of ropivacaine in serum and drainage blood.

	Results (range)	Mean ± SD
Knee arthroplasty patients (n = 20)		
Patient characteristics		
Sex (female/male)	12/8	
Age	60–85 years	73 ± 7 years
Weight	50–121 kg	87 ± 20 kg
Serum		
Total drug concentrations	0.23–1.5 µg/mL	0.64 ± 0.28 µg/mL
1. Sampling (2 h)		0.53 ± 0.21 µg/mL
2. Sampling (6 h)		0.69 ± 0.31 µg/mL
Drainage blood		
Total drug concentration	3.1–19 µg/mL	7.4 ± 4.3 µg/mL
Volume	50–620 mL	242 ± 140 mL
Total amount	0.28–12 mg	2.1 ± 2.6 mg
Hip arthroplasty patients (n = 10)		
Patient characteristics		
Sex (female/male)	4/6	
Age	56–87 years	72 ± 8 years
Weight	76–106 kg	85 ± 9 kg
Serum		
Total drug concentrations	0.30–1.1 µg/mL	0.62 ± 0.20 µg/mL
1. Sampling (2 h)		0.64 ± 0.20 µg/mL
2. Sampling (6 h)		0.58 ± 0.21 µg/mL
Free drug concentrations (n = 14)	0.012–0.068 µg/mL	0.036 ± 0.019 µg/mL
Free drug fractions (n = 14)	2.2–8.8%	5.7 ± 2.8%
Drainage blood		
Total concentration	2.8–11 µg/mL	7.0 ± 3.3 µg/mL
Volume	95–320 mL	196 ± 71 mL
Total amount	0.59–3.3 mg	1.4 ± 0.8 mg

may change the equilibrium of bound/unbound ropivacaine, thus for patient undergoing autotransfusion with ropivacaine it is both clinically relevant and interesting to study the free concentration. For this purpose the ultrafiltration technique is preferred for routine clinical laboratories. For ropivacaine in plasma ultrafiltration give equivalent or better results compared with microdialysis as shown by Koivisto et al. [28]. They used an ultrafiltration device (Ultrafree-MC, cutoff 30 kDa, Millipore) that is no longer available, but replaced with a similar type (see Section 2.6.2).

Nonetheless, to measure and compare data for free fractions of ropivacaine from patient samples collected over a period of time is an analytical challenge. Mathieu et al. reported 3.4–8.5% changes in free concentrations of ropivacaine after only 8 days of storage of serum samples and even after freezing at −20 °C [6]. Their findings also raise questions about the preparation of QC-samples and establishment of a target value for free ropivacaine. The present study showed an intra-assay precision of 2.8% (C.V.) in determination of free ropivacaine at a low total serum level and calculated free fraction percentages (Table 2) that correlated well with reference values [27]. However, handling and reanalysis of the small volumes of ultrafiltrates after freezing for four weeks at −18 °C introduced a systematic bias of about +5%, which requires that analysis of the free fraction should be performed extemporaneously as suggested by Mathieu et al. [6].

The project data are summarised in Table 2. Serum concentrations ranged from 0.23 to 1.5 µg/mL, which is in the same order of magnitude as hitherto reported after wound infiltration or epidural administration of ropivacaine [27]. Drainage volumes were also in agreement with previous observations [1,2]. In Parker et al.'s study drainage volumes varied from 300 to 700 mL (mean: 412 mL) and Stinger et al. reported drainage volumes ranging from 59 to 1015 mL in knee arthroplasty (mean 465 mL) and from 70 to 500 mL in hip arthroplasty (mean 233 mL). The total amount of ropivacaine in drainage blood found by Stinger et al. for the main study groups varied from 0.53 to 27.69 mg with mean values and stan-

dard deviation 10.44 ± 9.37 and 3.33 ± 1.87 mg for knee and hip arthroplasty, respectively [1]. These values are slightly higher than demonstrated in the present study (Table 2). However, Stinger et al. theoretically argued that post-surgery reinfusion of these amounts of ropivacaine should be safe.

Hitherto, only one study combining LIA with ropivacaine and blood reinfusion has been performed. Parker et al. [2] studied 20 otherwise healthy patients who received blood reinfusion with LIA after knee arthroplasty. Mean drain blood concentration of ropivacaine was 3.0 µg/mL (range: 0.58–4.77 µg/mL), mean total ropivacaine content in drainage blood was 1.3 mg (range: 0.4–2.6 mg) and serum concentration before reinfusion was 0.68 µg/mL (range: 0.26–1.20 µg/mL). These initial serum concentrations data are comparable with the knee arthroplasty data from the present study (Table 2), but the total amounts of ropivacaine in drainage blood are higher with maximum values of 12 and 3.3 mg for knee and hip arthroplasty, respectively. Thus it still needs to be investigated whether reinfusion of such amounts of ropivacaine in drainage blood is safe, before reinfusion in knee and hip arthroplasty can be introduced as a routine. Consequently, a reinfusion study is currently being planned by the authors.

4. Conclusion

A new, fast, accurate and precise HPLC–MS/MS method was developed for determination of ropivacaine in serum and drainage blood which is necessary to investigate and evaluate the safety of blood reinfusion after LIA in knee and hip surgery, which will have great benefits for patient safety and health economics. The advantage of the analytical method is a very simple and time-saving pre-treatment procedure, low sample volume and high specificity due to the absence of interference from co-extracted compounds and the use of tandem mass spectrometric detection. The column-switching technique itself is simple to implement in a routine laboratory. It is stable, robust and can be used as a generic template for other biomedical analytical methods and complex matrices.

References

[1] B.W. Stringer, A.K. Singhania, J.E. Sudhakar, R.B. Brink, J. Arthroplasty 22 (2007) 884.
[2] D.A. Parker, M.R.J. Coolican, L.E. Mather, D.A. Graham, M.J. DeWall, J. Arthroplasty 24 (2009) 918.
[3] E. Tanaka, T. Nakamura, S. Inomata, K. Honda, J. Chromatogr. B 834 (2006) 213.
[4] S. Reif, P. Le Corre, G. Dollo, F. Chevanne, R. Le Verge, J. Chromatogr. B 719 (1998) 239.
[5] A. Koehler, R. Oertel, W. Kirch, J. Chromatogr. A 1088 (2005) 126.
[6] O. Mathieu, D. Hillaire-Buys, C. Dadure, F. Barnay, J.C. Mathieu-Daudé, F. Bressolle, J. Chromatogr. B 831 (2006) 91.
[7] M. Stumpe, N.S. Morton, D.G. Watson, J. Chromatogr. B 748 (2000) 321.
[8] M. Abdel-Rehim, M. Andersson, E. Portelius, C. Norsten-Höög, L.G. Blomberg, J. Microcolumn Sep. 13 (2001) 313.
[9] Z. Cobb, L.I. Andersson, Anal. Bioanal. Chem. 383 (2005) 645.
[10] M. Engman, P. Neidenström, C. Norsten-Höög, S.-J. Wiklund, U. Bondesson, T. Arvidsson, J. Chromatogr. B 709 (1998) 57.
[11] M. Abdel-Rehim, K.A. Svensson, Y. Askemark, K.-J. Pettersson, J. Chromatogr. B 755 (2001) 253.
[12] M. Abdel-Rehim, J. Sep. Sci. 25 (2002) 252.
[13] S.K. Bergström, K.E. Markides, J. Chromatogr. B 775 (2002) 79.
[14] T. Arvidsson, E. Eklund, J. Chromatogr. B 668 (1995) 91.
[15] Z. Yu, M. Abdel-Rehim, D. Westerlund, J. Chromatogr. B 654 (1994) 221.
[16] Z. Yu, D. Westerlund, J. Chromatogr. A 725 (1996) 137.
[17] S. Emara, A. Khedr, H. Askal, Biomed. Chromatogr. 10 (1996) 131.

[18] Z. Yu, D. Westerlund, J. Chromatogr. A 725 (1996) 149.
[19] Z. Altun, M. Abdel-Rehim, L.G. Blomberg, J. Chromatogr. B 813 (2004) 129.
[20] Z. Altun, M. Abdel-Rehim, L.G. Blomberg, J. Mass Spectrom. 39 (2004) 1488.
[21] M. Abdel-Rehim, J. Chromatogr. B 801 (2004) 317.
[22] P. Sadílek, D. Ŝatínský, P. Solich, Trends Anal. Chem. 26 (2007) 375.
[23] L. Mondello, A.C. Lewis, K.D. Bartle (Eds.), Multidimentional Chromatography, John Wiley and Sons Ltd., 2002.

[24] J. Hermansson, A. Grahn, I. Hermansson, J. Chromatogr. A 797 (1998) 251.
[25] Z. Yu, D. Westerlund, Chromatographia 47 (1998) 299.
[26] A.N. Eeckhaut, K. Lanckmans, S. Sarre, I. Smolders, Y. Michotte, J. Chromatogr. B 877 (2009) 2198.
[27] K.J. McClellan, D. Faulds, Drugs 60 (2000) 1065.
[28] P. Koivisto, S.K. Bergström, K.E. Markides, J. Microcolumn Sep. 13 (2001) 197.