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On-line coupling of microdialysis to packed capillary column liquid chromatography–tandem mass spectrometry demonstrated by measurement of free concentrations of ropivacaine and metabolite from spiked plasma samples

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Abstract

An on-line coupling of microdialysis to a packed capillary column switching liquid chromatographic system (0.2 mm I.D.) and mass spectrometric detection was developed. The microdialysates were collected in the loop of the first of three valves, coupled in direct series. A deuterated internal standard was added on-line by the middle valve and the third valve operated both a pre-column, for desalting of the physiological buffer used in the sampling procedure, and a separation column. The on-line system was used to study free concentrations of ropivacaine and its metabolite (PPX) in human plasma samples. The analytes were detected by electrospray ionization in a tandem mass spectrometer operating in multiple reaction monitoring mode. The free fractions of ropivacaine (200 n*M* total concentration) and PPX (20 n*M* total concentration) in spiked plasma samples were 12±3 and 47±5% (±standard deviation for day-to-day variations, $n=3$), respectively. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Microdialysis; Ropivacaine

1. Introduction

Microdialysis is an advantageous sampling method for analytes from extracellular fluids since it can be coupled on-line to monitoring systems. In the sampling procedure, small molecules diffuse across a semi-permeable membrane into a perfusion liquid due to the concentration gradient, as reviewed by Lunte and Lunte [1]. To avoid disrupting the chemical balance, the optimal pH and ionic strength for sampling of blood should be kept at 7.4 and 0.2, respectively, i.e., the general physiological conditions. Commonly used perfusates are Ringers solution or a phosphate-buffered saline (PBS). Sampling of low concentration pharmaceuticals from plasma samples may require a sensitive detection method especially if the drugs and/or the metabolites are highly protein bound, since only the free fraction can diffuse across the membrane and since microdialysis sampling normally will provide less than 100% recovery. Mass spectrometric (MS) detection can provide the required sensitivity, but the high salt content in the physiological buffers are known to seriously disturb the ionization/vaporization process

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used for liquid-based MS detection. The coupling of microdialysis directly to MS or to liquid chromatography (LC)–MS is thus not always straightforward. Because of the non-volatile ions in the perfusate the curtain plate can require systematic cleaning to avoid loss of sensitivity [2]. Instead of a physiological buffer, a volatile buffer [3] or water [4,5], has been used as perfusate in some in vitro experiments. In other in vitro experiments, e.g., protein-binding studies and for in vivo sampling, it is essential to mimic the biological environment. The importance of physiological buffers and choice of buffer ions has been emphasized for equilibrium dialysis studies [6]. For on-line microdialysis studies, desalting before the MS ion source is thus recommended. A divert valve after the LC column [7] and a reversed-phase trap in a single column [8,9] can be used for desalting before MS detection. In the later cases the ion spray potential may need to be switched off in the washing step to avoid the salts to enter the mass spectrometer. A requirement for robust on-line microdialysis must include a back-pressure-free sampling process [10].

In this study, an on-line coupling of microdialysis to a column switching system including columns, of 0.2 mm I.D., is described. The detection was performed using electrospray ionization (ESI) MS detection. This system was applied to measure the free concentration of ropivacaine in plasma. The principle of column switching before MS detection, described by Swart et al., was modified for microdialysis applications [11]. A desalting pre-column and an internal standard was used to facilitate and control the ionization/vaporization process. A previous study reported a microdialysis method coupled on-line to a single packed capillary LC column (0.2 mm I.D.) and UV detection for determination of free concentrations of ropivacaine in small volumes (150 µl) of plasma [10]. In this work, the on-line microdialysis system was further developed for concentration levels in the lower part of the therapeutic interval of ropivacaine.

2. Experimental

2.1. Chemicals

All chemicals were of analytical grade if not otherwise stated. Disodium hydrogenphosphate, sodium dihydrogenphosphate, formic acid and anthracene, as well as methanol and acetonitrile of LiChrosolv grade, were obtained from Merck (Darmstadt, Germany). Ammonium formate was obtained from BDH (Poole, UK). Ropivacaine hydrochloride monohydrate, (*S*)-PPX (pipecoloxylidide) hydrochloride and deuterated ropivacaine hydrochloride monohydrate were obtained from AstraZeneca (Södertälje, Sweden). The structural formulas are illustrated in Fig. 1. Water was purified with a Milli-Q purification system (Millipore, Bedford, MA, USA).

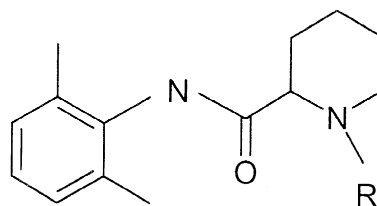

Fig. 1. The structural formulas for ropivacaine ($R=C_3H_7$), PPX ($R=H$) and deuterated ropivacaine ($R=C_3D_7$).

2.2. Standards and samples

A stock solution of 1000 µ*M* ropivacaine was prepared in water. Stock solutions of PPX and ropivacaine-D7 (355 and 476 µ*M*, respectively) were prepared in phosphate buffer, pH 2 (ionic strength 0.1). From these solutions, dilutions to 100 µ*M* of ropivacaine, PPX and ropivacaine-D7 were prepared in water and stored at +4 °C. Calibration standards were prepared from the 100 µ*M* stock solutions by dilution with a phosphate buffer, pH 7.4 (ionic strength 0.2). A single-point calibration at target concentration level (10 n*M*) was used. The concentration of internal standard was adjusted so its response was in the same range as for ropivacaine at the studied concentration level.

Human plasma was purchased from the blood bank at the University Hospital (Uppsala, Sweden). A few microliters (i.e. 5–10) of acetic acid (5%, v/v) was added to about 1 ml of plasma to adjust the pH to 7.4. The plasma was spiked to a total concentration of 200 n*M* of ropivacaine and 20 n*M* of PPX by adding 100 µ*M* stock solution. Prior to microdialysis, the plasma was incubated for 15 min at 37 °C in a block thermostat (DB-2D; Techne, Cambridge, UK) to assure protein binding, as in previous study [10].

Protein-free plasma for microdialysis probe calibration was prepared by ultrafiltration. Blank plasma was filtrated using an ultrafiltration device (Ultrafree 30.000, Millipore, Bedford, MA, USA) and a centrifuge (2K15, Sigma, Osterode am Harz, Germany) equipped with a 33° fixed angle rotor and a heating element. About 2 ml plasma was ultrafiltrated in each of two devices at 2000 *g* for 2×20 min at 37 °C to give about 900 μl filtrate. The filtrate was pH adjusted to pH 7.4 with acetic acid (5%, v/v) and spiked to a concentration of 10 n*M* of ropivacaine and PPX.

2.3. Preparation of separation- and pre-columns

The separation column was packed with 5 μm C_{18} particles (ODS A; YMC Europe, Schermbeck/Weselerwald, Germany) (pore size 120 Å) in a 0.2 mm I.D.×0.36 mm O.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) and used at a length of 145 mm. The column was packed using a slurry packing method. Acetonitrile was used both as slurry- and packing-solvent. About 20 mg packing medium was dissolved in 0.2 ml acetonitrile and sonicated to create the slurry. The slurry was transferred to a packing reservoir to which an empty capillary (200 mm) was connected. At the bottom of the capillary a piece of glass fibre paper (Whatman GF/A; W & R Balston, UK) functioned as a frit. The packing solvent transferred the particles into the column and packed them to a dense bed. The flow-rate of the packing solvent was 0.1 ml/min until the pressure reached 350 kg/cm^2, the pressure was then held constant at this level for about an hour. The column was tested to have an acceptable efficiency (>50 000 plates/m at the optimal flow-rate of about 1 μl/min) using anthracene and a mobile phase of acetonitrile–Milli-Q water (80:20, v/v).

A 10 mm long piece of a column prepared in the way described above was cut off to serve as a pre-column. At each end of the pre-column a piece of glass fibre paper (Whatman GF/A; W & R Balston) was inserted and served as a frit. The pre-column was placed inside two pieces of PTFE tubing (0.25 mm I.D.) and to each end of the pre-column a 70 mm (0.027 mm I.D.×0.36 mm O.D.) fused-silica was connected. The PTFE was fixed by a heat shrinkable tubing and to increase the mechanical strength of the pre-column a glass capillary (2 mm I.D.) (Schott Glaswerke, Mainz, Germany) was put outermost of the tubes and fixed with epoxy glue (Araldit rapid; CIPA, Switzerland).

2.4. Packed capillary LC column switching system

The mobile phases were A, methanol–ammonium formate, pH 3 (ionic strength 0.01) (5:95, v/v), and B, methanol–ammonium formate, pH 3 (ionic strength 0.01) (50:50, v/v). Two LC pumps (PU-980, Jasco, Tokyo, Japan) were used for the delivery. The packed capillary column switching system contained three valves. The first and the middle valve were manual injectors; a six-port valve with 2.5 μl external sample loop (C6W, Valco instruments, Houston, TX, USA) and a four-port valve with 60 nl internal loop (C14W, Valco). The third valve was an electrically actuated six-port switching valve (Valco, Schenkon, Switzerland). The pre-column construction was connected to the third valve, where the loop normally is situated. The separation column was integrated to this valve as well. All valves were coupled together by two fused-silica capillaries (0.095 mm I.D.×0.36 mm O.D.), each at a length of 15 cm. The column switching system is shown in Fig. 2.

Standards or microdialysates and internal standard were injected in the first and middle valves, respectively. These two valves were switched at the same time and mobile phase A loaded both the sample and the internal standard on the pre-column at a flow-rate of 20 μl/min. The loading time was optimized to 50 s, based on the breakthrough volume of PPX (the least retained compound). The third valve was then switched and the pre-column was back-flushed by mobile phase B allowing the analytes to be transferred to the separation column. The same mobile phase was used for eluting the separation column and the second LC pump was operating in constant pressure mode at a flow-rate of 1 μl/min. While analyzing one sample the system allowed simultaneous sampling of the consecutive sample. After the third valve had been switched, to back-elute onto the separation column, the first injector was left in inject position for at least 1 min before it was switched back to load position.

The chromatography was performed at ambient temperature.

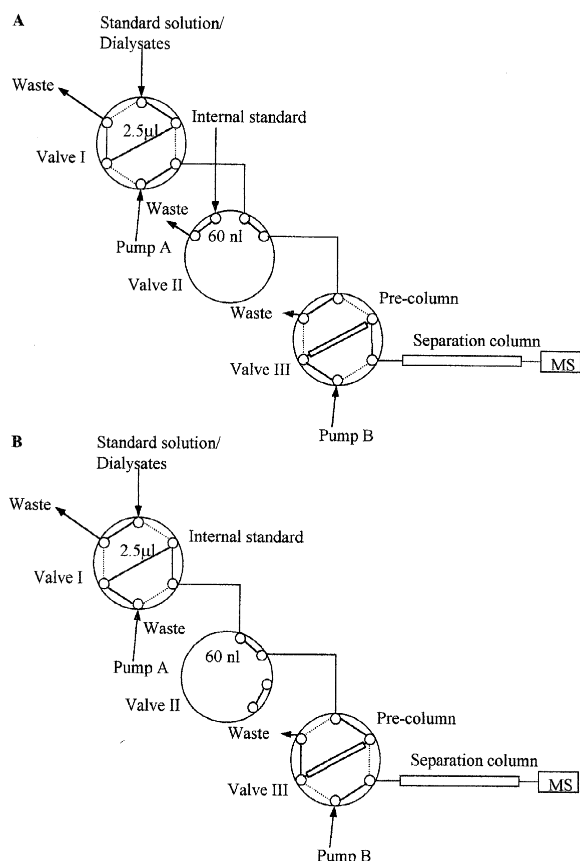

Fig. 2. The column switching system for collection of microdialysates in valve I, addition of internal standard in valve II and desalting in valve III. (A) While analyzing one sample the consecutive sample and internal standard were loaded simultaneously. Valves I and II were in load positions and the pre-column in valve III was back eluted to transfer analytes to the separation column. (B) When loading analytes and internal standard to the pre-column, valves I and II were switched at the same time and compounds were flushed to the pre-column (valve III in load position).

2.5. Off-line versus on-line collection of microdialysates

Off-line versus on-line collection of microdialysates were compared in a study with probes built in the laboratory. The membrane material in the probes was polyamide with 20 kDa molecular mass cutoff (MWCO) at a length of 8–9 mm. The recovery was compared for ropivacaine and PPX in a 1.0 μ*M* solution in phosphate buffer, pH 7.4, ionic strength=0.2 (150 μl sample volume) in a study concerning recoveries at different flow-rates (0.1–2 ml/min). The same probe was used in both the off-line and the on-line study and two measurements were made for each flow-rate. The dialysis time for the different flow-rates was adjusted to get a proper injection volume.

2.6. Microdialysis sampling of free concentrations

Commercial microdialysis catheters (CMA 70 brain-microdialysis catheter; CMA, Stockholm, Sweden) equipped with a 20 mm long polyamide membrane with 20 kDa MWCO were used. A catheter was placed in 200 μl sample solution and perfused with phosphate buffer, pH 7.4 (ionic strength 0.2) using a CMA/102 microdialysis pump from CMA (Stockholm, Sweden) equipped with a 1 ml microsyringe. The polyurethane outlet tube was replaced by a fused-silica (125×0.10 mm I.D.) capillary by a tight fit and coupled on-line to the LC system. The resulting dead volume from the membrane to the injector was 3.1 μl. Microdialysis samples were collected in the loop of the first valve for 25 min, at a flow-rate of 0.5 μl/min.

The relative recovery of the microdialysis probe was daily checked by comparing the concentration in the microdialysates with the concentration in an ultrafiltrated and spiked plasma sample at 10 n*M* level. The plasma was ultrafiltrated before the spiking to remove the drug binding proteins. A new sample (200 μl) was used for each measurement to avoid the risk for depletion of the analyte. A total analysis time of 25 min was chosen to adjust for the dead volume from the probe to the injector when changing samples and to properly fill the injection loop before injection.

Free concentration determinations of ropivacaine and PPX in the spiked plasma samples was performed on 3 different days. The microdialysis probe was pre-treated in plasma (during perfusion) for at least 30 min before it was used in the spiked plasma sample. Three consecutive samples were analysed without removing the probe. These measurements only sampled the free fraction which is in equilibrium with the protein bound fraction. The risk for depleting the sample is thus much lower in this sampling compared to the recovery sampling.

2.7. Mass spectrometry

Mass spectra were recorded on a PE-Sciex API 365 triple quadrupole mass spectrometer (PE-Sciex, Concord, Canada). The electrospray was performed using a sheathless Micro Ion interface. The separation column was connected on-line to the interface via a fused-silica capillary (300×0.05 mm I.D.) which further was linked to the spray capillary (57×0.015 mm I.D.) by a low-dead volume stainless steel union. The tip of the spray capillary was unmodified and positioned approximately 15 mm in front of the orifice and 8 mm off axis.

Mass spectrometric parameters, such as spray voltage, interface potentials, probe position and gas flows, were optimized during direct infusion of a 0.5 μ*M* solution of the analytes dissolved in mobile phase B. A flow-rate of 1 μl/min was delivered by a syringe pump from Harvard Apparatus (Holliston, MA, USA). The optimized ion spray voltage and orifice potential was 4.2 kV and 30 V, respectively.

Tandem mass spectrometry, in multiple reaction monitoring (MRM) mode, was used for all analyses. The m/z values for the mother–daughter ion pairs studied in the first and third quadrupole (Q1 and Q3) for PPX, ropivacaine and ropivacaine-D7, were 233–84, 275–126 and 282–133, respectively.

Chromatograms were recorded with a dwell time of 100 ms. The electrospray stability as well as the exact m/z values for the analytes in Q1 and Q3 was daily controlled during infusion of 0.5 μ*M* solution of the analytes in mobile phase at a flow-rate of 1.0 μl/min.

The linearity of the LC–MS system was checked by injecting serial diluted (1:10) solutions in the concentration range of 0–1.0 μ*M*. All analytes were injected in the first valve (2.5 μl loop volume).

3. Results and discussion

The on-line coupling of microdialysis sampling to an analytical system is time saving, a prerequisite for automation and a suitable way to handle and chemically protect the analytes from, e.g., oxygen and light, during the analysis. Such a system also allows a time-resolved analysis. In the study concerning on-line versus off-line sample collection at different flow-rates it was found that the recovery increases at lower flow-rates. This was expected since the time for diffusion into the perfusate is thereby also increased. Further, and more surprisingly, a paired *t*-test of the mean recoveries at the different flow-rates showed a significant higher recovery ($P<0.05$; $n=7$) for on-line collection compared to off-line collection for both ropivacaine and PPX. Also the repeatability was indicated to be better for the on-line collection when studying the pooled variances of the two methods for the different flow-rates. However, this tendency could not be confirmed with an *F*-test at the 5% significance level. Together, these results illustrate some additional advantages of using on-line sample collection.

The high salt content inherent in the microdialysates can more or less disturb the detection depending on what kind of detector that is used. In the previously reported microdialysis–LC–UV system [10], the detection sensitivity was not affected by the high salt level while severe signal suppression was expected and evident in an initial microdialysis–LC–MS study without desalting and addition of internal standard. The on-line system described in the present work, with a packed capillary column switching arrangement, was found to be a useful tool in the MS detection and to markedly prolong the lifetime of the separation column.

3.1. On-line microdialysis–LC–MS

In this study, all steps from the sampling of a biological fluid to the MS detection are integrated in an on-line system. The microdialysis conditions optimized in the previous work [10] was applied also in this study. This included minimizing the back-pressure from the outlet tube and the injector and effective enrichment of the dilute dilaysate. The polyurethane outlet tube was here exchanged into fused-silica to minimize the dead volume to the first valve. In order to get an analytical system of medical grade, this study employed a commercial microdialysis probe containing a polyamide membrane. This provided a system and a method that in the future, when miniaturized mass spectrometers are further developed, can even be used for in vivo sampling in a patient followed by direct on-line analysis.

The on-line system was constructed with low dead volumes, considering the length and internal diameter of the transfer tubes. A rather large sample volume (2.5 μl) of high-ionic-strength perfusion buffer was collected in the first loop of the three on-line valves. The content was injected simultaneously with the internal standard, in the second valve, onto the pre-column for enrichment and desalting. The pre-column gave pre-concentration of the analytes and was also essential for desalting the sample prior to mass spectrometric analysis. Salts and polar compounds that otherwise could disturb the ionization/volatilization process were thus washed out of the system by the high flow-rate. The pre-column model described here was found to be very mechanically stable. Due to the high loading flow-rate the pre-column had to withstand rather high pressures and the external glass capillary was found to add the needed stability and to increase the lifetime of the construction. Different models of pre-columns were investigated and the main problem was leakage in the PTFE tube connections. The pre-columns had to withstand a pressure of about 55 kg/cm^2, which is the pressure in the separation column and the pressure the pre-column is exposed to during the back-elution. At least four pre-columns, without external glass capillary, leaked before 25 injections were made. The pre-column model with external glass capillary was used during the whole study and was still functional after 200 injections. The desalting process was satisfactory, giving stable spray and good sensitivity, and the analytes were transferred into a mobile phase compatible with ESI-MS detection. No deposits that lowered the sensitivity were observed and there was also no need for regular cleaning of the entrance to the mass analyzer. As a comparison, a 40% decrease in signal was seen in an initial screening.

The use of a deuterated internal standard may be the most efficient way of decoupling variations in the ionization/volatilization process from the MS response. In this study, the addition of internal standard was performed after the microdialysis sampling since its function was to compensate for variations in the electrospray process and therefore it had to be independent of the variations in the sampling procedure. This system set-up thus required on-line addition of the internal standard to the microdialysates. A valve with small internal loop volume (60 nl) was placed between the sampling valve and the analytical valve. A small loop volume was used to minimize the extra volume added, but the correct injection of such a small volume is critical and may give an additional contribution to the variations in the system. The relative standard deviation (RSD) for injection of a 10 nM standard solution in phosphate buffer of ropivacaine and PPX (injected in the first valve) evaluated with internal standard (injected in the middle valve) could be as high as 10% ($n=3$, using height ratio between analyte and internal standard evaluated). The precision for the addition of internal standard is expected improved by using a larger internal loop volume. Loop volumes of 200 nl will thus be used in further studies.

In microdialysis, small volumes of samples are collected and if the analyte concentrations in the samples are low, which is common when studying drugs that are prone to protein binding, the choice of analytical techniques are limited. Packed capillary column LC is suitable since the chromatographic dilution is reduced in these columns giving increased sensitivity if a concentration sensitive detector, e.g., UV or ESI-MS, is used. A further advantage is that the volume of non-volatile perfusate buffer injected to the LC–MS system is small, decreasing the risk for deposits on the MS-curtain plate.

A simple separation of the analytes was performed since a fragment from deuterated ropivacaine could affect the detection of PPX. Ropivacaine and deuterated ropivacaine had, as expected, the same retention time ($t_R=13.7$ min) and PPX was just separated from them by a few minutes ($t_R=11.2$). Fig. 3 shows a total ion chromatogram as well as extracted ion chromatograms of a spiked and microdialyzed plasma sample. The signal for a blank plasma sample was below the limit of quantification (LOQ), corresponding to the analyte concentration giving a peak height of 10 times the standard deviation of the noise.

The LC–MS system showed good linearity from 0.1 to 100 nM (R^2 were 0.9999 for all analytes). The curve equations ($n=4$ points) from the linearity study, with 95% confidence intervals for the slope and intercept for the different analytes, were $y=87.6(\pm1.06)x-8.15(\pm53.23)$, $y=22.2(\pm0.16)x-0.45\ (\pm8.22)$ and $y=70.1(\pm0.84)x-8.20(\pm42.39)$

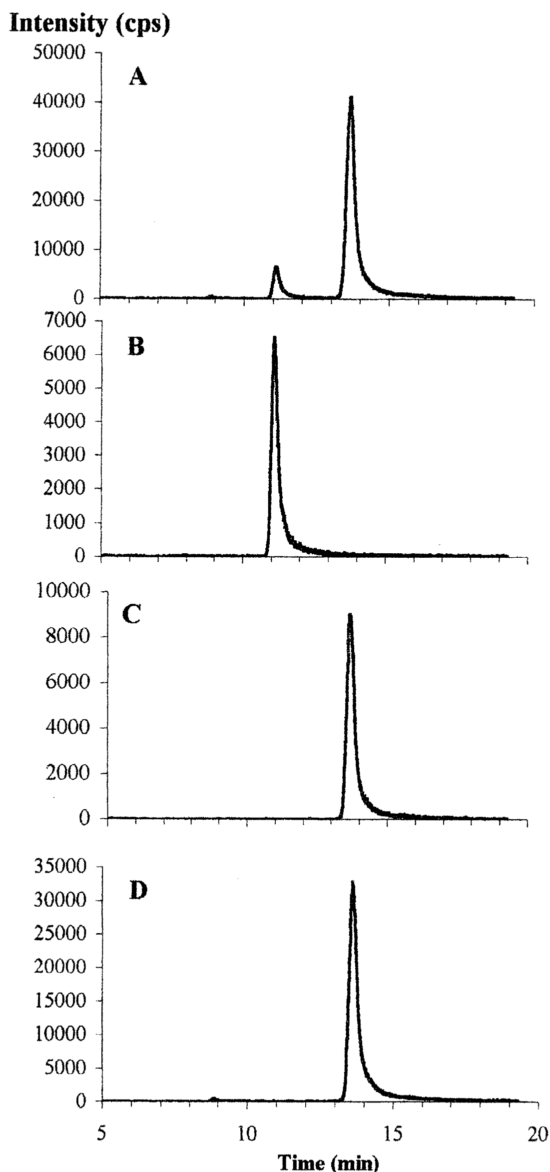

Fig. 3. A typical chromatogram of a plasma sample spiked to a total concentration of 200 n*M* ropivacaine and 20 n*M* PPX. From the total ion chromatogram (A) the *m*/*z* values for the daughter ions created were extracted for each analyte (B–D). PPX (B), with *m*/*z* 84, was just separated from ropivacaine (C), *m*/*z* 126, and deuterated ropivacaine (D), *m*/*z* 133, which had the same retention times.

for ropivacaine, PPX and ropivacaine-D7, respectively (y=peak height in counts per second$\cdot 10^{-2}$ and x=analyte concentration in n*M*). The LOQ was found to be 0.1 n*M* for all analytes (peak height from chromatograms obtained by injecting all analytes in the first valve was evaluated). The single point calibration was used within the linear range at target concentration to adjust the slope for the day. The day-to-day variation of the slope of the calibration curve was found to be 15% for PPX and 8.2% for ropivacaine (n=3 days).

3.2. Free concentration analysis

In the previous free fraction study, plasma was spiked to 4.0 μ*M* and the resulting free concentration of ropivacaine was then about 240 n*M*. Free concentrations of ropivacaine are pharmacologically interesting to measure down to 10 n*M* in pharmacokinetic [12] and adverse effect studies [13]. The PPX concentration in a blood sample from a patient is expected to be less than 10% of the ropivacaine concentration [14]. In this work, plasma was thus spiked to a total concentration of 200 n*M* of ropivacaine and 20 n*M* of PPX in order to develop a method for low therapeutic levels realized by MS detection. The concentration of the single point calibration standard was chosen as 10 n*M* of both target drug and metabolite from the expected free fractions. A single point calibration, well within the linear range, was chosen to get a quick quantification process of the unbound drug. This decreased the total analysis time, which otherwise was quite long since each dialysis took about half an hour. The precision obtained from such a calibration is however not as good as for an ordinary calibration curve based on several calibration points but it was found sufficiently enough in this initial study of technology development to study the level of unbound drugs.

The recovery of the microdialysis probe was evaluated in the matrix that appeared most similar to real plasma: ultrafiltrated and spiked plasma. At 10 n*M* level the recovery was found to be about 50% for ropivacaine and about 67% for PPX with standard deviations (SDs) for the within sample measurements of 4.8 and 8.8%, respectively (n=3). (Peak height ratio for analyte and internal standard was used for the evaluation). The recovery was checked daily and could differ between probes from day to day. To get a measurement of the free concentration,

the average of at least three determinations of the recovery per day, was used to correct the measured concentration.

The free concentrations were evaluated from the calibration curve and corrected for the probe recovery. For ropivacaine and PPX the free fractions were found to be 12±3 and 47±5% [average±SD for day-to-day variations (n=3)], respectively. Consequently, the degree of protein binding was found to be lower for the more polar metabolite PPX, compared to ropivacaine. Earlier studies has reported the free fraction of ropivacaine to be 1–8% [15–17]. The earlier reported free fractions of ropivacaine were thus lower than 12% found here, but none of the previous studies were performed at such low concentration levels as in this work. A more probably reason for higher values would be variations in the α_1-acid glycoprotein (the protein that dominates the ropivacaine protein binding) concentration. The protein concentration is individual and may be altered if the concentration of plasma proteins suddenly change, which may be the case in surgery, pregnancy and certain diseases [18]. It is thus possible that the protein concentration varies and in this case it would be important to measure the free concentration of drugs bound to these proteins, instead of the total, to provide better dosing. Ropivacaine would still, at a free fraction of 12%, be classified as a highly bound drug. The measured free fraction of 47% for PPX was, however, in closer agreement with earlier studies [14].

The drug protein binding was indicated to be relatively fast, since no trends for decreasing free concentration within the three repeated measurements were found. The drug–protein binding kinetics should, however, be studied in more detail to draw further conclusions about this theory.

Within the measurements for 1 day the average SD for the free fraction was as high as 2% for ropivacaine and 3% for PPX (corresponding to RSD values of 17 and 11%, respectively). The relatively large variations in the results can be explained by a combination of many factors. As already shown in the recovery studies, it is difficult to perform microdialysis at low concentration levels with good precision. At this low concentration level, small variations in the free concentration give large variations in the free fractions and also the sensitivity to unwanted and uncontrolled adsorption to tubes increases. The high inter-assay precision for ropivacaine indicates that there may be differences in protein binding of this drug for different aliquots of a plasma sample. Some of the variations can also be explained by the variation in addition of internal standard as discussed above.

A phosphate buffer with, pH 7.4 and ionic strength 0.2 is a simple way to mimic the conditions in plasma. This perfusate was found to be useful for measurements of free concentrations of ropivacaine and PPX. Other drugs that are more sensitive to the perfusate ion composition require a more complex perfusate. The system described in this work can, however, be applied to microdialysis sampling with perfusates of various compositions.

4. Summary

The combination of microdialysis with the capillary column LC system, coupled to tandem mass spectrometric detection, is an on-line integration of sampling and detection demonstrated by the determination of free concentrations of ropivacaine and its metabolite in spiked plasma. This system is approaching an in vivo system where mass spectrometric detection is needed: a physiological buffer, to mimic the biological environment and a microdialysis probe of medical grade, was thus used. A capillary pre-column provided effective on-line desalting of the high ionic strength perfusate and the on-line addition of internal standard was further controlling variations in gas-phase ion formation.

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