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Determination of talinolol in human plasma using automated on-line solid phase extraction combined with atmospheric pressure chemical ionization tandem mass spectrometry

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Abstract

A specific LC–MS/MS assay was developed for the automated determination of talinolol in human plasma, using on-line solid phase extraction system (prospekt 2) combined with atmospheric pressure chemical ionization (APCI) tandem mass spectrometry. The method involved simple precipitation of plasma proteins with perchloric acid (contained propranolol) as the internal standard (IS) and injection of the supernatant onto a C_8 End Capped (10 mm × 2 mm) cartridge without any evaporation step. Using the back-flush mode, the analytes were transferred onto an analytical column (XTerra C_{18}, 50 mm × 4.6 mm) for chromatographic separation and mass spectrometry detection. One of the particularities of the assay is that the SPE cartridge is used as a column switching device and not as an SPE cartridge. Therefore, the same SPE cartridge could be used more than 28 times, significantly reducing the analysis cost. APCI ionization was selected to overcome any potential matrix suppression effects because the analyte and IS co-eluted. The mean precision and accuracy in the concentration range 2.5–200 ng/mL was found to be 103% and 7.4%, respectively. The data was assessed from QC samples during the validation phase of the assay. The lower limit of quantification was 2.5 ng/mL, using a 250 μL plasma aliquot. The LC–MS/MS method provided the requisite selectivity, sensitivity, robustness accuracy and precision to assess pharmacokinetics of the compound in several hundred human plasma samples.

Keywords: Talinolol; Quantitative analysis; Column-switching; LC–MS/MS

1. Introduction

Talinolol (Cordanum®) a racemic, highly selective, β_1-adrenoceptor antagonist has been used in once daily dosages as a therapy for arterial hypertension and coronary heart disease, in Germany and Eastern Europe, for many years [1,2]. To support pharmacokinetic studies in man, various assays were developed, mainly based on normal phase or reversed phase separation followed by UV, fluorescence or mass spectrometric detection. Oertel et al. [3] described an LC-UV assay where basified serum was extracted with diethyl ether. Metoclopramide was used as the internal standard (IS). The analyte was separated on a normal-phase column and the detection was performed at 254 nm. The assay was found to be linear in the range 5–200 ng/mL using a 1 mL serum aliquot. The same group [4] achieved better sensitivity using mass spectrometric detection in the selected reaction monitoring (SRM) mode with ion spray ionization in the positive mode. Using a serum aliquot of 0.2 mL, a LOQ of 200 pg/mL could be achieved. The analyte was isolated from serum using solid phase extraction. Reversed phase liquid chromatography followed by fluorimetric detection (252 nm extinction, 332 nm emission) was also applied to determine talinolol in serum [5]. The assay was found to be linear in the range of 5–1000 ng/mL using a 0.5 mL serum aliquot. The analyte was isolated from serum using liquid–liquid extraction with diethyl ether and separated on a reversed phase standard bore column, using propranolol as the internal standard.

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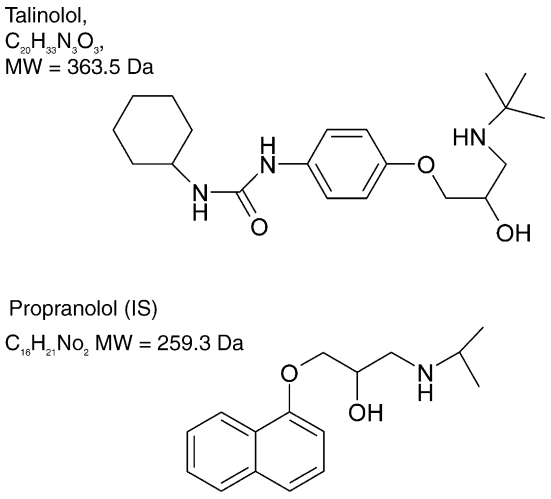

Fig. 1. Structure of talinolol and propranolol–HCl.

In bioanalysis LC–MS/MS has become the method of choice for the quantification of pharmaceutical compounds in biological fluids [6]. With LC–MS/MS, sample preparation remains an important step and its automation greatly improves sample throughput. To support formulation studies an assay was developed for the quantitation of talinolol in human plasma using an on-line sample preparation column-switching approach, with the prospekt 2 device. To enhance the robustness of the assay, protein precipitation with perchloric acid was applied as a sample pre-treatment.

The prospekt system (Spark Holland) is an automated 96-cartridge on-line solid phase extraction device [7,8]. Typically the diluted biological fluid (urine, plasma or serum) is injected onto an extraction cartridge. After the washing steps the cartridge is placed on-line (front-flush or back-flush mode) with the chromatographic system. In general, for each new analysis, a new cartridge is used [8,9]. The major advantage compared to off-line solid phase extraction is that no evaporation of the extract is required.

In the present work, the prospekt 2 device is not used as a regular on-line SPE device, but as a column-switching device, where the same cartridge is run numerous times. Therefore, prospekt's cartridges were not used as SPE cartridges but rather as trapping columns. An assay using automated on-line solid-phase extraction with atmospheric pressure chemical ionisation (APCI) tandem mass spectrometry is described in this paper for the quantification of talinolol, using propranolol (Fig. 1) as the internal standard.

2. Experimental

2.1. Reagents and solvents

Ultra pure Water (for chromatography) was produced in house using a Milli-Qplus 185 from Millipore (Volketswil, Switzerland). Methanol (MeOH), acetonitrile (HPLC grade), and perchloric acid (70%, p.a.) were obtained from Merck (Darmstadt, Germany). Acetic acid (ca. 98%, puriss p.a.) and ammonium acetate (purum p.a.) were purchased from Fluka (Buchs, Switzerland). Talinolol and propranolol–HCl were obtained from the clinical toxicology and pharmacology department of Basel Hospital (Switzerland). Ammonium acetate buffer (5 mM) was made from ammonium acetate (0.384 g) dissolved in de-ionized water (1000 mL) and adjusted with 1% (v/v) of acetic acid.

Calibration and quality control (QC) stock solutions were obtained by dissolving the pure test compound and internal standards in methanol. Stock solutions could be stored at 5 °C for about six months. Working solutions were prepared by diluting aliquots of the stock solutions with water at appropriate concentrations to prepare human plasma standards. Working solutions were freshly prepared and could be stored at 5 °C for about a month.

The blank plasma required for preparation of the standards consisted of a pool of plasma, using EDTA as the anticoagulant. Human calibration and QC plasma standards were obtained by spiking blank plasma with an appropriate volume of working solution providing plasma talinolol concentrations between 2.5 and 200 ng/mL. Plasma standards were then divided into aliquots and stored deep-frozen at −20 °C until required for analysis.

2.2. Sample preparation-work-up

Plasma proteins were precipitated by adding to the sample (250 μL), of a perchloric acid (0.5 M) solution containing propranolol as the internal standard (250 μL, 200 ng/μL). After neutralization with ammonium acetate 1M (500 μL), the sample was centrifuged (ca. 14,000 × *g*, 10 min). A 500 μL aliquot of the supernatant was then injected onto the system.

2.3. Chromatographic system—instrumentation

The complete analytical system (Fig. 2) consisted of two liquid chromatographic pumps (Model 10 Advp; Shimadzu, Rupperswill, Switzerland) operated in high pressure gradient mode and the prospekt 2 system from Spark Holland (Emmen, The Netherland) equipped with a dual high pressure dispenser unit, and a dual automated cartridge exchange unit. Sample injection was performed with a triathlon autosampler using an injection volume of 500 μL. Chromatographic separation was achieved at 800 μL/min using a 50 mm × 4.6 mm, 3.5 μm i.d. XTerra MS analytical column (AC) (Waters, Rupperswil, Switzerland), which was preceded by a 4 mm × 2 mm C18 phenomenex precolumn filter (Brechbuehler AG, Switzerland). The mobile phases consisted of (A1) 5 mM ammonium acetate in water + 1% acetic acid and (B1) 5 mM ammonium acetate in methanol + 1% acetic acid. The HPLC gradient program is listed in Table 1.

2.4. Analytical procedure

The on-line column-switching procedure is listed in Table 2. The cartridges were conditioned with 1 mL of

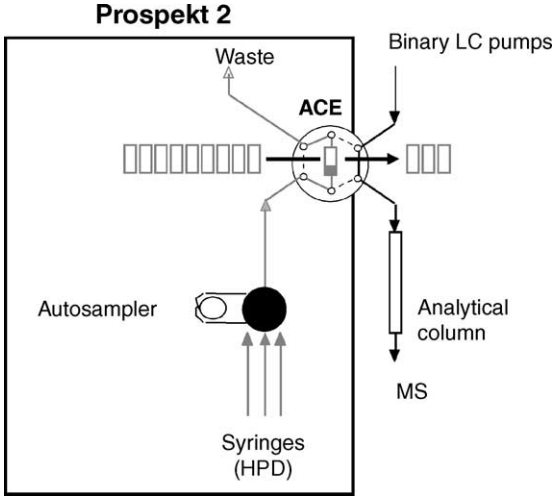

Fig. 2. Schematic representation of the analytical setup (see text for details). Prospekt 2 device is composed of an autosampler (Triathlon), a dual syringe high-pressure dispenser (HPD) and an automatic cartridge exchange (ACE) module.

methanol in order to wet the packing material, and subsequently conditioned with 1 mL of 5 mM ammonium acetate in water + 1% acetic acid in order to obtain suitable conditions for analyte adsorption. Next, 500 μL of the supernatant was loaded onto the cartridge using 1 mL of 5 mM ammonium acetate in water + 1% acetic acid. As a final sample preparation step, the cartridge was washed with a 0.1 mL mixture of 5 mM ammonium acetate in water + 1% acetic acid/5 mM ammonium acetate in methanol + 1% acetic acid (70:30, v/v). All steps mentioned thus far were performed at a flow rate oscillating between 2 and 5 mL/min. After completion of the trapping phase, the cartridge was switched on-line for 80 s to desorb the analyte and transfer it through an analytical column, directly into the mass spectrometer.

Table 1
HPLC gradient program

Time (min)	% Solvent B
0.1	30
2.0	90
2.5	90
2.6	30
4.0	30

Solvent A1: 5 mM ammonium acetate in water (+1% acetic acid, v/v), Solvent B1: 5 mM ammonium acetate in MeOH (+1% acetic acid, v/v).

Table 2
Time schedule for the analytical procedure—Prospekt program

Time (min)	Step	Flow rates (μL/min)	Action
0	New cartridge		
0.10	Solvation	5000	1000 μL Solvent D
0.22	Equilibration	5000	1000 μL Solvent A
1.22	Sample injection	2000	1000 μL Solvent A
1.52	Wash (H_2O)	1000	2000 μL Solvent A
2.16	Wash (MeOH)	2000	100 μL Solvent C
2.19	Elution	800	80 s transfer on AC
3.40	End		

Solvent A: 5 mM ammonium acetate in water (+1% acetic acid, v/v), Solvent B: 5 mM ammonium acetate in MeOH (+1% acetic acid, v/v), Solvent C: A/B 70/30 (v/v), Solvent D: MeOH, AC: analytical column.

2.5. Mass spectrometry

A triple quadrupole linear ion trap mass spectrometer (QqLIT) (QTRAP: AB/MDS Sciex, Concord, Canada) equipped with a heated nebulizer source with corona discharge was used, with the following settings: curtain gas 30 psi; temperature 475 °C; gas 1: 60 psi; gas 2: 30 psi; declustering potential: 20 eV. The whole effluent (0.8 mL/min) of the analytical column was therefore introduced into the source using nitrogen both as nebulizer and curtain gas. Q1 and Q3 resolutions were set to give a 0.7 Da FWHM (full width at half maximum) for both precursor and product ion.

The selected mass-to-charge (*m*/*z*) ratio transitions of the protonated analyte and internal standard ions $[M + H]^+$ used in the selected reaction mode (SRM) were as follows: (I) talinolol, *m*/*z* 364.1–*m*/*z* 100.3 Th; (II) propranolol, *m*/*z* 260.1–*m*/*z* 183.2 Th. The dwell times were set at 100 ms for the analytes and at 250 ms for the internal standard. All ions were monitored in a single period. For collision induced dissociation (CID), nitrogen was used as collision gas at a setting of 3 (laboratory frame). The collision energy for fragmentation of the precursor ions was set at 30 and 20 eV for talinolol and propranolol, respectively. The absence of the hydroxylated metabolites in study samples was screening in SRM mode using the following transitions: *m*/*z* 380.1–*m*/*z* 308.1 and *m*/*z* 380.1–*m*/*z* 100.1 Th.

2.6. Method validation

For method validation, the QC samples were prepared at a low concentration (less than three times the LLOQ) the expected lower limit of quantification (LLOQ), a mid range, an upper range concentration and the upper limit of quantification (ULOQ), respectively 2.50, 3, 20, 150 and 200 ng/mL. Samples were extracted and analyzed on five different events. Intra- and inter-variability, accuracy, LLOQ and stability were assessed to determine the validity of the bioanalytical method [10]. The selectivity (the absence of interferences from endogenous components in the biological matrix or exogenous components from the isolation procedure) was assessed by extracting control blank plasma samples in each validation run. The lack of interfering peaks at the same analyte retention time was considered as acceptable selectivity.

Stability calculations of talinolol were as follows: after storage, the samples were analyzed with an equal number of freshly prepared samples to provide the 100% values. The experimental procedure involved replicate analysis of the drug in stored and freshly prepared samples on the same day. The relative difference in response between these two sets of samples and a 90% confidence interval for the true change in re-

sponse was then calculated. This confidence interval enabled us to detect relevant degradation [11].

The analyte recovery (including extraction and suppression) was determined by comparing the absolute peak area of the spiked plasma versus the peak area of the analyte in the injection buffer.

2.7. Calibration and calculation

A Dell PC computer was used for instrument control, data acquisition and data processing. Data acquisition and integration of SRM chromatograms were performed running the Analyst software packages (Version 1.3.2) from AB/MDS Sciex (Concord, ON, Canada).

Along with the biological samples at least seven plasma standards (2.50, 10, 25, 50, 100, 150, 200 ng/mL) covering the expected concentration range were processed. The standard curves for the analytes were obtained by weighted least-squares regression (weighting = $1/x^2$) of the measured peak area ratios analyte/internal standard versus the analyte concentrations added to the plasma. The standard curves were then used to calculate concentrations of the analytes in unknown and QC samples from the measured peak area ratios.

3. Results and discussion

3.1. Sample clean-up and liquid chromatography

Direct plasma analysis is highly desirable and has also been investigated for the quantitation of talinolol. However, with classical cartridges (7 μm particle size) a new cartridge is required for each new injection, otherwise severe pressure increase after three or four injections was observed. With direct plasma injection, the addition of the internal standard can also be considered to have a sample pre-treatment step, because it generally requires vortexing and centrifugation of the sample. To increase robustness of the assay and enhance the lifetime of the cartridge, a protein precipitation step using perchloric acid was selected. The perchloric acid solution also contained the internal standard. This procedure was already successfully applied on different hydrophilic analytes [12,13]. A requisite to using perchloric acid is to ensure that the compound of interest is stable in acidic conditions. Protein precipitation with perchloric acid over organic modifiers (ethanol, acetonitrile) offers the advantage that no evaporation of the sample to dryness is required. Limiting sample evaporation is important because it has been observed that thermal degradation or adsorptive loss can cause partial loss of the analyte.

Compared to a regular on-line column-switching device, the prospekt 2 system offers a wide range of cartridge sorbents and the versatility of changing a cartridge automatically whenever necessary. With the protein precipitation approach more than 90 injections could be performed on a single cartridge without loss of performance. For routine sample analysis the cartridge was changed automatically after about 28 injections. This means that the calibrations sample and the QC's samples were analyzed on different cartridges without affecting assay performance. Compared to direct plasma injection the multiple injection of the protein precipitate supernatant, on a single cartridge significantly reduced cost and increased assay robustness. A key advantage of column-switching compared to other approaches is that with column-switching the injection volume can be adjusted to the sensitivity need of the assay.

In bioanalysis, method development is also a time intensive step. With classical column-switching, only one trapping column can be used. With a column selector the number of trapping columns can be increased to six or eight columns. The prospekt can hold different trapping columns, therefore part of the method development can be performed automatically overnight [14]. A limitation is that the columns have a fixed length. Various sorbents (C_2, C_8, C_8 End Capped, C_{18}, C_{18} End Capped, C_{18} high density and polymeric phase) cartridges were tested. Due to a good peak shape and an acceptable retention time of our analytes, the C_8 EC cartridge was selected. The transfer of the analyte from the trapping column to the analytical column is a critical step in column-switching. The time in which both columns are on-line has to be set carefully. If the time is set too short, poor recovery of the analyte is observed. If the time is set too high, many endogenous compounds can be transferred to the analytical column, which will affect the long-term performance of the assay. A transfer time of 80 s in the back-flush mode was found to be ideal for the assay.

To increase the speed of the assay, a dual, high-pressure dispenser system was used. While aspirating solvents from one syringe, the other dispenser was delivering another solvent on the cartridge. An entire cycle lasted 4.8 min per sample (13 samples per hour, 150 samples per 12 h).

For quantitative analysis, stable, isotopically labeled internal standards are preferred because they compensate for losses during the sample manipulation steps and suppression effects during ionization. Propranolol cannot be considered as an optimal IS and to anticipate potential problems the selection of the chromatographic system was performed in such a way that the analyte and the IS co-elute. Gradient elution was also selected to wash out endogenous compounds from the analytical column after each analysis. Autosampler carry over was also investigated and not found to be critical for the assay. No carry-over was observed when a blank solution was injected onto the cartridge after injecting the highest calibration point.

3.2. Mass spectrometry considerations

Preliminary trials already indicated that talinolol and propranolol were equally amenable to sensitive detection using either ionspray or the heated nebulizer interface. The product ion spectra of talinolol and propranolol are illustrated in Fig. 3. Matrix effects during the ionization process, resulting in signal suppression or enhancement caused by the

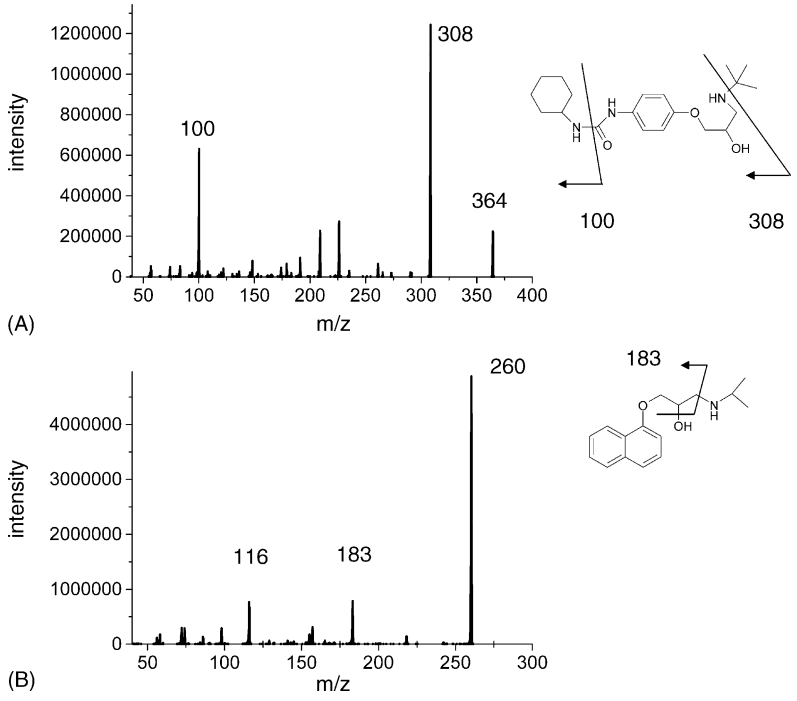

Fig. 3. MS/MS spectra of (A) talinolol 30 eV collision energy. (B) Propranolol 20 eV collision energy.

co-elution of endogenous compounds such as salts, amines, fatty acids, or triglycerides, is a commonly accepted phenomena with atmospheric pressure ionization. These effects are strongly compound dependent and are also more pronounced with electrospray ionization than with atmospheric pressure chemical ionization [15]. Therefore, owing to the robustness of the heated nebulizer interface at high flow rates and less ion suppression, the assay was developed with the heated nebulizer interface. The optimal temperature was found to be 475 °C with the eluent used at a flow rate of 800 μL/min. For talinolol two possible product ions (*m*/*z* 308 and *m*/*z* 100) can be considered. The fragment at *m*/*z* 100 was selected because it was considered to be the most selective compared to the loss of 56 Da.

The original intention of the assay was to use calibration samples made up in EDTA plasma to analyze EDTA or Li-Heparin plasma samples. Only the QC's would have been prepared in the respective plasma type. However, different analyte/IS ratios (factor of 2) were obtained for the different anticoagulants. Therefore, a unique standard curve in EDTA or Li-Heparin plasma could not be used to calculate concentrations of the analytes in unknown EDTA and Li-Heparin samples. Mei et al. [15] have investigated the effect of various Li-Heparin concentrations on mass signal intensities and found a significant matrix suppression effect for some of the analytes investigated.

3.3. Linearity and limit of quantification

For talinolol, the therapeutic plasma concentrations in humans rarely exceeded a value of 100 ng/mL [16] and, for this reason, a single standard curve could include all concentrations from human plasma. The calibration range was selected according to the concentrations anticipated in the samples to be determined. The concentration range 2.50–200 ng/mL proved to be sufficient. The lower limit of quantification was set at 2.50 ng/mL to meet the criterion for the precision and inaccuracy of less than or equal to 20% and 15%, respectively. If required, better sensitivity can be achieved by using a more sensitive triple MS instrument or by increasing the injection volume up to 2 mL.

3.4. Matrix suppression

Matrix suppression or enhancement during the ionization process can be a severe problem when developing LC–MS assays [17]. One of the fundamental issues in bionalysis is that method validation is performed with spiked matrix samples. Despite that the same matrix, plasma for example, is used for preparing calibration and QC samples, significant differences can be observed between these samples and the study samples. It is therefore important to identify possible suppression or enhancement issues and several approaches have been proposed [17]. One of the approaches is to investigate the suppression in various lots of plasma. In column-switching assays it is challenging to directly determine suppression. However, the parameter which can be easily determined, is the combination of extraction recovery and suppression (overall analyte recovery). In the present work the overall analyte recovery for the plasma was found to be 66% for talinolol (40 ng/mL, $n = 5$, CV = 8%) and 82% for propranolol (40 ng/mL, $n = 5$, CV = 6%). APCI was selected be-

cause compared to ion spray it is generally less prone to matrix suppression. Also the chromatographic parameters were optimized in such a way that the analyte and the internal standard were voluntarily co-eluting. Study samples from different subjects may behave differently regarding matrix suppression.

The absolute peak area of the propranolol (IS) was used as a marker to monitor possible suppression effects with study samples. The RSD of propranolol peak area was found to be less than 6% for batches of more than 80 samples including samples from different subjects, calibration and QC samples. It was therefore concluded that the matrix suppression for this assay, is not an issue.

3.5. Selectivity

The intercepts of the calibration graph did not significantly differ from zero, underlining the selectivity of the assay. Based on the analysis of drug free plasma from control plasma and blank samples included in each validation run, endogenous or chemical components did not interfere with the drug and internal standards over the concentration range described herein (Fig. 4). Analysis of study pre-dose samples did not show any significant interference.

Talinolol has very little metabolism [18]. In humans the major metabolic pathway of talinolol is hydroxylation of the cyclohexyl ring [3] and the metabolites were detected in human urine. Phase II metabolites such as glucuronides or sulfate metabolites were not reported. Reference compounds were not available and the various hydroxylated metabolites of talinolol were generated by incubation of talinolol in rat hepatocytes. The metabolites were found to co-elute with talinolol and a SRM-enhanced product ion experiment was performed to screen the presence of the metabolites in study samples. Various study samples were re-analyzed randomly and no metabolite signal was detected in any of them. For the assay, potential metabolite cross-talk, in the quantification of talinolol was not found to be critical.

The triple quadrupole linear ion trap used in this work can be simultaneously run in quadrupole or trap mode. With this instrument it is possible to acquire an SRM experiment and obtain an enhanced full scan product ion spectrum (EPI) with similar sensitivity. The combination of SRM and EPI mode was used to confirm that no endogenous compounds or metabolites are interfering with the quantitation of talinolol [19].

3.6. Intra–inter-assay precision and accuracy

The precision and the accuracy were evaluated for therapeutic concentration range in human plasma. Intra–inter-assay precision and accuracy were evaluated by assaying QC samples prepared in human plasma and are summarized in Table 3. Typically with the prospekt a new extraction cartridge would be used for each new analysis. This avoids any potential carry-over from one analysis to another but results in relatively high consumable cost. In generally, the packing material of the cartridge is of relatively good quality and several injections should be possible on the same cartridge in particular when protein precipitation is applied as sample pre-treatment. From our experience it was found that 28 injections, can be performed easily on the same cartridge without loosing retention performance. When in an analytical sequence multiple cartridges are used special attention has to be given for method validation and analysis of study samples. In the current configuration calibration samples were always analyzed on the first cartridge and when a second or third cartridge was necessary the sequence was set in such a way that additional QC samples were always run on the different cartridges. The mean precision and accuracy, calculated from QC samples at all three levels (3, 20 and 150 ng/mL $n = 32$) during analysis of study samples was found to be 7.5% and 103%.

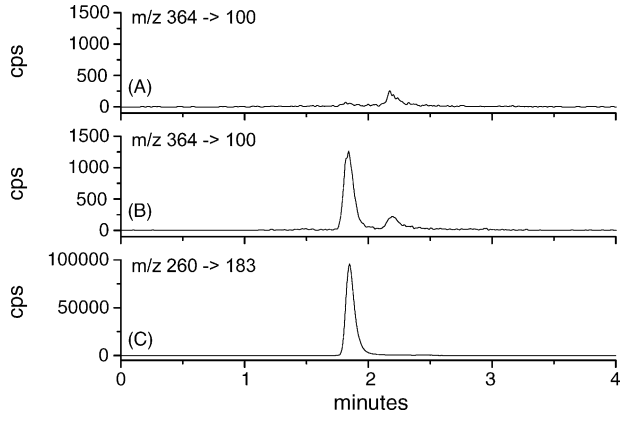

Fig. 4. Representative selected reaction monitoring chromatograms of talinolol in human plasma (A) plasma blank, (B) calibration sample at LLOQ 2.5 ng/mL and (C) propranolol IS.

Table 3
Intra-assay (A) and inter-assay (B) precision and accuracy for talinol in human plasma derived from quality control samples ($n = 5$)

Concentration (ng/mL)	Mean	Standard deviation	RSD (%)	Accuracy (%)
(A)				
2.50	2.52	0.13	5.2	101
3.00	3.25	0.07	2.0	108
20.0	20.3	0.55	2.7	102
25.0	25.4	0.40	1.6	102
150	151	1.64	1.1	101
200	208	5.97	2.9	104
(B)				
2.50	2.61	0.10	3.7	105
3.00	3.29	0.04	1.2	110
20.0	20.4	0.37	1.8	102
25.0	26.1	0.58	2.2	105
150	154	2.61	1.7	102
200	213	4.04	1.9	107

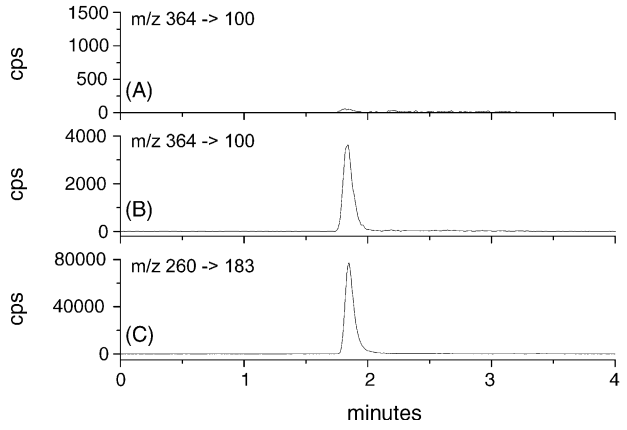

Fig. 5. Representative selected reaction monitoring chromatograms of talinolol (A) predose sample, (B) 48 h after ingestion up-take corresponding to 3.63 ng/mL of talinolol and (C) propranolol IS.

3.7. Stability in human plasma

Human EDTA plasma was spiked with talinolol and propranolol at concentrations of 5 or 50 ng/mL and 200 ng/mL respectively and stored for different time intervals at 25 and −20 °C. The statistical interpretation of the data followed acceptance criteria stated by Timm et al. [11]. Talinolol was found to be stable at 25 °C for at least 24 h and for a period of 11 months at a temperature of −20 °C in EDTA plasma. Talinolol was also stable in the autosampler at room temperature for at least 24 h.

3.8. Application to biological samples

The practicability of the new assay has been demonstrated by the analysis of approximately 560 patient plasma samples from a clinical study [20]. This assay has been used for the determination of talinolol in plasma following gastro intestinal incubation administration to assess the pharmacokinetics in humans. Fig. 5 displays the SRM chromatograms of talinolol and its internal standard propranolol from plasma samples taken before administration and 48 h following nasogastric administration of 50 mg of talinolol in 125 mL of aqueous solution delivered at a flow rate of 5 mL/min. The concentration was found using a 250 μL plasma aliquot. Fig. 6 depicts a representative plasma concentration–time profile for talinolol.

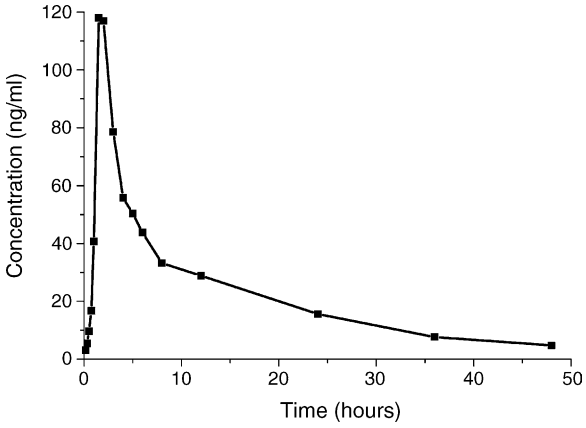

Fig. 6. Plasma concentrations vs. time curves from a representative patient. The subject received 50 mg of talinolol via a nasogastric infusion.

4. Conclusions

An automated LC–MS/MS method has been developed for determination of a specific βblocker, talinolol, in human plasma utilizing on-line solid phase extraction and detection by APCI using the SRM mode. Prior to analysis the plasma proteins are removed by protein precipitation using a perchloric acid solution. Sample preparation is therefore simple and not labour intensive. The method was validated in the concentration range 2.5–200 ng/mL. Except for the plasma precipitation step, the assay was completely automated, allowing unattended operation. The applicability of this LC–MS/MS assay using the prospekt 2 device with multiple injections on single cartridges was demonstrated for mapping of complete concentration–time profiles to assess pharmacokinetic parameters in humans.

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