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Short communication

Determination of articaine in human plasma by liquid chromatography–mass spectrometry and its application in a preliminary pharmacokinetic study

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ABSTRACT

A specific liquid chromatography–mass spectrometric (LC–MS) method using an ion trap spectrometer was developed for the quantitation of articaine in human plasma. Articaine and the internal standard (trazodone) were extracted in a single step with diethyl-ether from 0.5 mL of alkalinized plasma. The mobile phase consisted of acetonitrile with 0.1% formic acid (40:60, v/v). It was delivered at a flow rate of 0.3 mL/min. The effluent was monitored by MS in positive-ion mode. Ionisation was performed using an electrospray ion source operating at 200 °C. Articaine was identified and quantified in SIM mode at *m/z* 185. Calibration curves were linear over the concentration range of 78.1–5000 ng/mL with determination coefficients > 0.996. This method was fast (total run-time < 3 min), accurate (bias < 16%), and reproducible (intra-assay and inter-assay precision < 14%) with a quantitation limit of 78.1 ng/mL. The good specificity and sensitivity achieved by this method allowed the determination of articaine plasma levels in patients following a submucosal infiltration injection of articaine in the patients undergoing a third molar surgery.

1. Introduction

Articaine hydrochloride [4-methyl-3(2-propylaminopropionamido)thiophene-2-carboxylic acid methyl ester hydrochloride, MW = 320.836] is a local anesthetic agent. Articaine is classified as an amide but contains a thiophene ring instead of a benzene ring like other amide local anesthetics [1]. A second molecular difference between articaine and other amide local anesthetics is the extra ester linkage incorporated into the articaine molecule, which results in hydrolysis of articaine by plasma esterases. Isen states that 90–95% of articaine is metabolized in the blood whereas only 5–10% is broken down in the liver [2]. Articaine hydrochloride, commonly found as a 4% solution containing epinephrine 1:200,000, is widely used in France in dentistry for infiltration and regional anesthesia [3,4]. The main reasons of its wide utilization are a high quality of anesthesia, a reduced systemic toxicity and a short duration of action [1,5].

Third molar surgery is in the commonest procedures undertaken in oral and maxillofacial surgery units [6]. For most patients, the removal of all four wisdom teeth must be done during the same surgical procedure. Thus, one-stage procedure has potential advantages, chiefly among which is the avoidance of a second procedure, the decreased overall anxiety, the reduction in time taken off work and general disruption to daily life for the patient. General anesthesia is often suggested. Previous works have shown the relevance of local anesthesia alone or with intravenous sedation as an alternative general anesthesia to the extraction of four third molars [6,7].

So far, only two HPLC methods are available to measure articaine in human serum or plasma. They are based on either a serum protein precipitation [8] or a solid-phase extraction procedure [9]. The limits of quantitation were of about 10–100 ng/mL, and the methods were capable of measuring both articaine and its metabolite, articainic acid. Both of theses methods were appropriate for pharmacokinetic studies of low dose submucosal injections of articaine in dentistry.

In this work, we propose an alternative assay method for the determination of articaine levels in human plasma. Particular attention has been paid to optimize the method for both specificity and speed of the analytical run. To this end, a fast, specific LC technique coupled to ion trap MS was developed to identify and quantify articaine in human plasma. After validation, this method was used in a preliminary clinical study monitoring the plasma concentrations after repeated injections in patients undergoing wisdom teeth removal.

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2. Experimental

2.1. Reagents

Articaine hydrochloride was supplied by PIERRE ROLAND Laboratory (France) (chemical structure in Fig. 1). Trazodone hydrochloride (internal standard (IS)) was purchased from SIGMA (Saint-Quentin Fallavier, France) (chemical structure in Fig. 1). Organic solvents and reagents were all of LC grade. Acetonitrile and diethyl-ether were supplied by SDS (Peypin, France). Methanol and formic acid were obtained from Merck (Darmstadt, Germany). Purified water was prepared on a Milli-Q Waters purification system (Millipore, Saint-Quentin en Yvelines, France).

2.2. Biosamples

Blank human plasma samples were supplied from our local blood bank. Authentic blood samples were collected from patients included in a clinical study approved by the local Ethic Committee, University Hospital of Reims, France.

2.3. Standard solutions and calibration curves

Stock standard solutions of articaine and its IS were prepared in methanol at a concentration of 1 mg/mL, and stored at +4 °C. These were further diluted in methanol to give appropriate working solutions used to prepare the calibration solutions. Standard curves were prepared in human plasma (1 mL) to yield final concentrations of 78.1, 156.2, 312.5, 625, 1250, 2500 and 5000 ng/mL.

2.4. Sample preparation

Plasma sample (500 μL) was extracted with 5.0 mL of diethyl-ether after addition of 10 μL IS solution (1.0 mg/L trazodone in methanol) and 200 μL of carbonate buffer (Na_2CO_3 20%, pH 9.0). The mixture was vortex mixed for 1 min, and then centrifuged at 3000 × *g* for 5 min. The organic layer was transferred into conical glass tubes and evaporated to dryness under a nitrogen stream at 40 °C. The residue was finally dissolved in 200 μL of acetonitrile:0.1% formic acid (40:60, v/v), and 20 μL were injected into the LC column.

2.5. Liquid chromatography–mass spectrometry

2.5.1. Equipment and chromatographic conditions

The LC–MS^n system consisted of a Thermofinnigan Surveyor® LC system (Les Ulis, France) equipped with an autosampler. Compounds were screened for, identified, and quantified in plasma using a Thermofinnigan LCQ Advantage® trap ion mass spectrometer, and the Thermofinnigan Xcalibur® data system. Chromatographic separations were carried-out by using a 5 μm particle size Hypurity C18 column (150 mm × 2.1 mm I.D., ThermoHypersil-Keystone, Les Ulis, France) whose temperature was maintained at 30 °C. Samples were eluted isocratically with a mobile phase consisting of acetonitrile:0.1% formic acid in purified water (40:60, v/v) delivered at a flow rate of 0.3 mL/min. The entire flow was directed into the source without splitting. During use, the mobile phase was degassed by an integrated Surveyor® series degasser. In order to optimize the MS parameters, infusion experiments were carried-out with a 500 μL syringe connected to a pump with a flow rate of 5 μL/min.

2.5.2. Mass spectrometry conditions

The ionisation technique used was electrospray ionisation (ESI) in the positive-ion mode for both compounds. The spray needle was set at a potential of 4 kV. The heated capillary was set at 200 °C, and the stainless-steel capillary held at a potential of 10 V. Nitrogen was used as drying and nebulising gas. The sheath gas flow rate of nitrogen was set at 40 (arbitrary units). The tube lens offset was set at 40 V and the electron multiplier voltage set at 400 V peak-to-peak. Ultra-pure helium (99.995%) was used in the trap as damping and collision gas. The instrument was set to acquire three microscans, and ion injection time into the trap was optimized by using the integrated automatic gain control software.

2.5.3. MS conditions for identification and quantitation

The detection of articaine and trazodone was performed by LC–MS^n in different MS mode. Three alternating scan events were set up as follow:

(1) for trazodone, fragment ions of the molecular ion were generated through collision-induced dissociation (CID) at *m*/*z* 372 (protonated molecular ion $[M+H]^+$). Full scan MS–MS spectra was produced using a normalized collision energy of 50%. The reference MS–MS spectra of trazodone was previously collected using direct injection *via* the integrated syringe pump. Once full mass spectra of the product ions were generated, post-acquisition data processing was designed to select particular ions for quantitation, usually, fragment ions with the greater intensity (i.e. *m*/*z* 176, Fig. 1);
(2) for articaine, SIM mode was employed. The *m*/*z* ratio 285 $[M+H]^+$ was recorded for quantification;
(3) for confirmation of the identity of articaine, another fragment (*m*/*z* 168) was monitored by applying a fragmentor voltage of 35 V.

Peak-area ratios of the target ion of articaine (*m*/*z* 285) *versus* that of the IS (*m*/*z* 176) were compared with calibration curves prepared under the same conditions. If drug concentrations in authentic samples exceeded the calibration range, samples were reanalyzed after appropriate dilution with drug-free plasma.

2.5.4. Calculations

The calibration curves were calculated by weighted least-squares linear regression analysis (weight was 1/concentration) of the concentrations of the analyte *versus* the peak-area ratio of the target ion for quantitation of articaine to that of the IS. Concentrations of unknown samples were determined by applying the linear regression equation of the standard curve to the unknown sample's peak-area ratio.

2.6. Method validation

2.6.1. Quality control

Quality controls were prepared from a pool of blank human plasma spiked with three different amounts of articaine corresponding to the LOW, MEDIUM, and HIGH concentrations given in Table 1. Plasma aliquots were stored at −20 °C until assayed and were renewed every 3 months.

2.6.2. Precision and accuracy

Precision and accuracy of the assay were assessed by replicate analysis of quality control samples of articaine. Fifteen and thirty separate samples were assayed for intra- and inter-day evaluations, respectively. Precision expressed as relative standard deviation (% R.S.D.) was expected to be <15% except at the limit of quantitation (LOQ) where 20% was acceptable. Accuracy (bias) was determined as [100 × (mean measured concentration/spiked concentration) − 100]. Bias of ±15% was considered satisfactory, except at the LOQ where ±20% was acceptable.

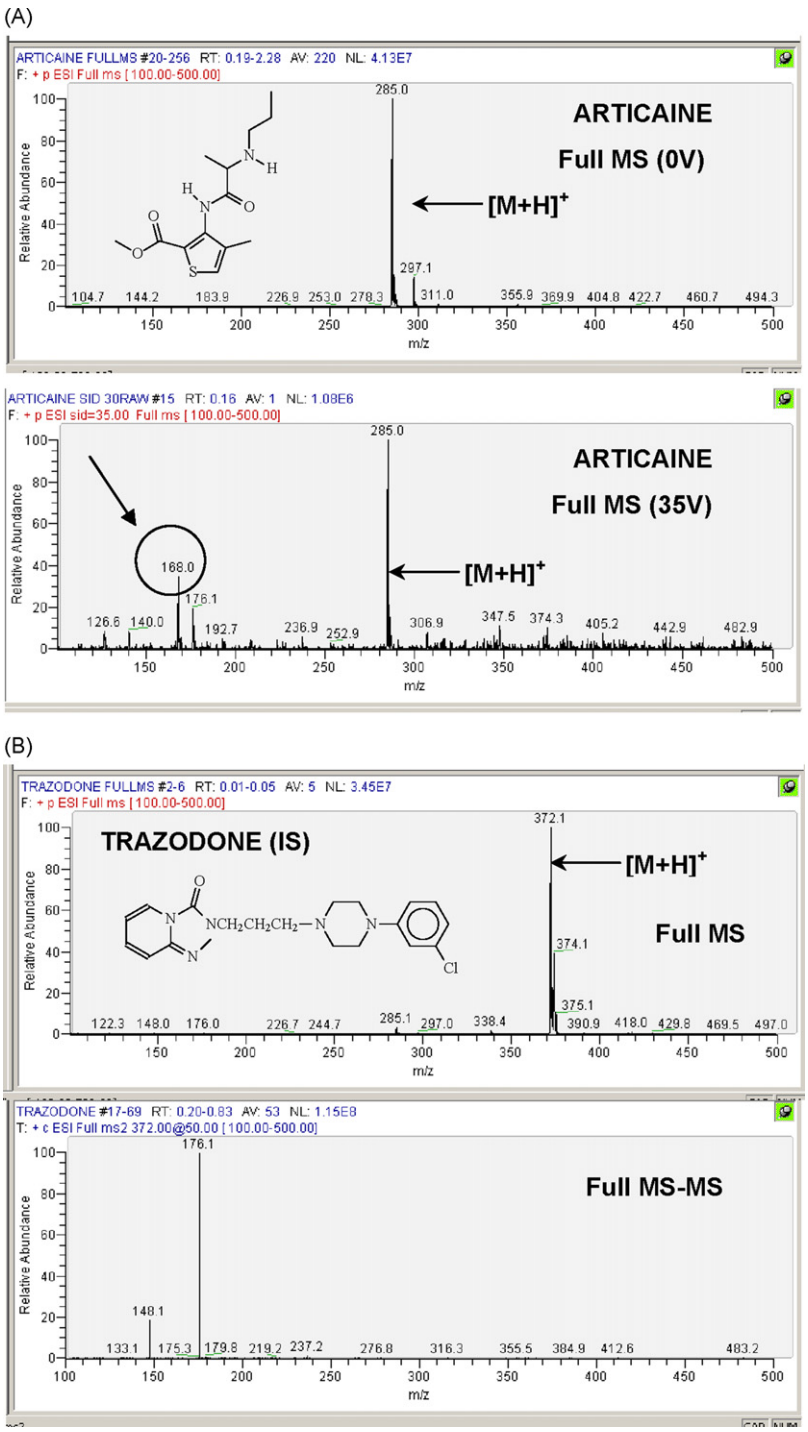

Fig. 1. (A) ESI–MS recorded at 0 and 35 V fragmentor voltage of articaine; (B) ESI–MS and ESI–MS/MS spectra of trazodone (IS). *x*-axis: *m*/*z* values; *y*-axis: relative abundance.

Table 1
LOD, LOQ, linearity, intra-day ($n = 15$) and inter-day ($n = 30$) precision and accuracy of the LC–MS assay for articaine. Extraction recoveries of articaine in human plasma.

Analyte	LOD (ng/mL)	LOQ (ng/mL)	Linearity (ng/mL)	Spiked LOW MEDIUM HIGH (ng/mL)	R.S.D.[a] (%)		Bias[b] (%)		Extraction recovery		
					Intra-day	Inter-day	Intra-day	Inter-day	Concentration (ng/mL)	(%)	R.S.D.[a] (%)
Articaine	9.8	78.1	78.1 –5000	78.1	6.4	11.6	−15.5	−7.3	156	42	3.9 ($n = 5$)
				625	6.3	10.1	−4.1	−11	1250	40	6.8 ($n = 5$)
				2500	5.1	13.3	−11	3.4	5000	42	4.7 ($n = 5$)

[a] Relative standard deviation.
[b] Expressed as [100 × (mean measured concentration/spiked concentration) − 100].

2.6.3. Limits of detection and quantitation

The limit of detection was determined from the detector response after direct injection of decreasing amounts of articaine. A signal-to-noise ratio of 10 was taken as the limit of detection. A signal-to-noise of at least 20 was taken as the LOQ. Criteria for the LOQ were fulfilled by the lowest point of the calibration curve.

2.6.4. Carry-over

The lack of carry-over effect was assessed by alternately analyzing blank plasma samples ($n = 3$) and plasma samples containing concentrations at the upper limit of quantitation of each compounds ($n = 3$). The residual concentration found in the first blank plasma sample following a high concentration sample was used to calculate the rate of carry-over. It was considered minimal if below 0.5% of the LOQ.

2.6.5. Extraction recoveries

Extraction recoveries from human plasma were evaluated at three concentrations levels: 156, 1250 and 5000 ng/mL ($n = 5$). The samples were extracted without IS according to the procedure described above. 10 μL of IS solution (1 mg/L trazodone in mobile phase) were added to the organic phase, and evaporated to dryness. The residue was dissolved in 150 μL of mobile phase prior to analysis. As controls ($n = 5$), articaine solution in mobile phase at the two concentrations levels to which were added 10 μL of IS solution were gently evaporated. The residues were then dissolved in 150 μL of mobile phase and analyzed. Recoveries were calculated by comparing peak areas of controls to those of spiked plasma samples.

2.6.6. Specificity and ion-suppression test

The specificity of the method was evaluated by analyzing 10 different plasma samples obtained from healthy volunteers who did not receive articaine. The ion-suppression effect of the method was also assessed with these plasma samples. After extraction, they were injected in the LC–MS–MS system while continuous post-column infusion of concentrations of 1000 ng/mL of articaine and IS (flow rate of 5 μL/min) was in effect, as described by Müller et al. [10]. Since articaine and trazodone elute at a closed time, additional ion-suppression experiments were carried-out to check for a potential mutual ion-suppression effect of these two compounds. Briefly, extracted samples containing a LOW concentration of either articaine or trazodone were injected in the LC–MS–MS system with and without an additional HIGH concentration of the corresponding compound ($n = 3$).

2.6.7. Method application

This technique has been applied to measure articaine concentrations in plasma samples collected from six patients enrolled in a clinical study. Each volunteer received, on two separate occasions (at $T = 0$ min and $T = 30$ min after the first injection), 238 mg of articaine hydrochloride (3.7 mL of 4% articaine with 1:200,000 epinephrine; corresponding to 211 mg of articaine; PRIMACAINE Adrénaline 1.200 000®, PIERRE ROLAND, MERIGNAC, France) administered as repeated buccal infiltrations. Blood were collected before and at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90, 120 and 180 min after the first injection of articaine. Immediately after withdrawal, blood samples were cooled in ice-cold water and plasma was separated by centrifugation. This was done because, as previously reported, cooling blood samples to 4 °C substantially reduces the activity of plasma esterase [9]. All samples were rapidly frozen and stored at −20 °C until analysis.

3. Results and discussion

3.1. Mass spectrometry and chromatography

ESI source was preferred over the atmospheric pressure chemical ionisation source since the latter resulted in bad ionisation of the compounds being studied under our experimental conditions. Similarly, positive-ion mode was chosen in order to obtain the most intense signal of the molecular cation ($[M+H]^+$). Fig. 1 represents the mass spectra of articaine and IS. For articaine, SIM mode was preferred over full MS–MS scan mode (using the protonated molecular ion $[M+H]^+$ m/z 285 as a precursor ion, and a normalized collision energy varying from 10 to 50%) because the latter produced a low and unstable signal. As proof, repeated analysis ($n = 10$) of the same sample containing articaine (1000 ng/mL in mobile phase) showed coefficients of variations of the peak areas obtained of 16 and 3.8% following analysis in full MS–MS mode and SIM mode, respectively. Because SIM mode is less specific than full scan MS–MS mode, the fragment ion m/z 168 was monitored as a confirmation ion by applying an "in source" fragmentation voltage of 30 V.

For quantitation purposes, trazodone was chosen as an IS because this compound is not marketed in France and hence was not administered in patients included in the ongoing clinical study. Although no reciprocal ion suppression (see below) occurs during the co-elution of the both compounds, other chromatographic standards eluting at a different retention time of that of articaine could be evaluated. For instance, the use of a deuterated internal standard such as diazepam-d5 with a retention time of 2.4 min may certainly be an advantage over using trazodone, and could be considered for this assay.

Fig. 2 shows reconstructed ion chromatograms (RIC) of a blank plasma spiked with IS and MEDIUM concentration of articaine. Under our analytical conditions, articaine is not chromatographically separated from its IS, with retention times of 1.70 min for both articaine and trazodone. However, due to the good selectivity of MS, complete chromatographic separation is theoretically not necessary any more; it is nevertheless important to check for the absence of mutual ion-suppression effect (see specific paragraph below). By using an isocratic elution of compounds, total run-time was shorter than 3 min per sample. For application to pharmacokinetic studies, the single-step extraction procedure combined to a short chromatographic run-time could be considered as an important benefit.

3.2. Validation data

3.2.1. Precision and accuracy

Table 1 summarizes mean values, precision and accuracy of intra- and inter-assay analyses. Precision and accuracy were within acceptable ranges for bio-analytical purposes. Intra-day precision ranged from 5.1 to 6.4%, and accuracy (bias) was less than 10%, except for the limit of quantification. Inter-day precision did not exceed 16% over the three quality control samples investigated. The accuracy of the technique was considered satisfactory since the between-day bias over the concentration range studied was found to be in the range −7.3 to 3.4%.

3.2.2. Linearity, limits and carry-over

Calibration curves for articaine in human plasma exhibited good linearity over the concentration range studied (i.e. 78.1–5000 ng/mL). Using weighted linear regression analysis, they were best described by the following equation: $Y = 0.0889X + 0.00207$ ($r^2 = 0.998$), where Y is the peak-area ratio of compound of interest to IS and X is the plasma concentration. Values of the coefficients of determination were all >0.996.

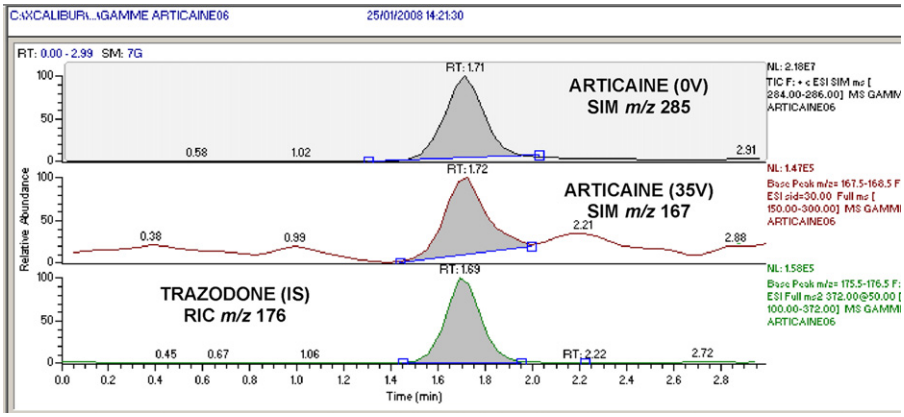

Fig. 2. LC–ESI–MS (SIM mode recorded at 0 and 35 V fragmentor voltage for articaine) and MS–MS smoothed ion chromatograms (reconstructed ion chromatogram; RIC) of a blank plasma spiked with IS (trazodone) and articaine at a concentration of 625 ng/mL.

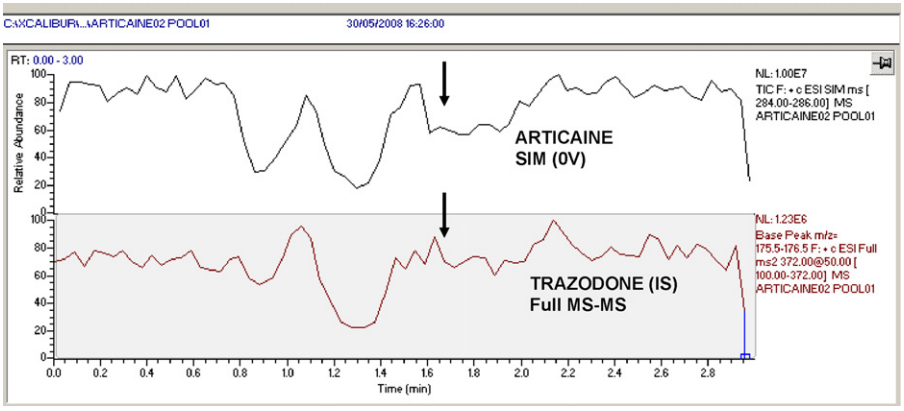

Fig. 3. Results of ion suppression for articaine and IS (trazodone) assayed in positive ionisation mode. Continuous post-columns infusion of all the compounds with on-column injection of blank plasma extract. No ion suppression was observed after elution of the LC front (retention time > 1.5 min).

LODs are reported in Table 1. The LOQ for articaine in plasma was chosen as the lowest calibration standard concentration (78.1 ng/mL) for which the CV and bias did not exceed 20 and ±20%, respectively. These LOQ is higher than the one described by Richter and Oertel (10 ng/mL from 1 mL of plasma sample) [9]. It should be noted that the method reported by Richter and Oertel was developed for pharmacokinetic studies with submucosal injection of a low articaine hydrochloride dose (up to 16 mg) on humans [9]. As shown below, the developed method showed a LOQ that is sufficient for the purpose of a pharmacokinetic study of articaine in patients following a submucosal infiltration injection of more than 200 mg of articaine hydrochloride in repeated buccal infiltrations.

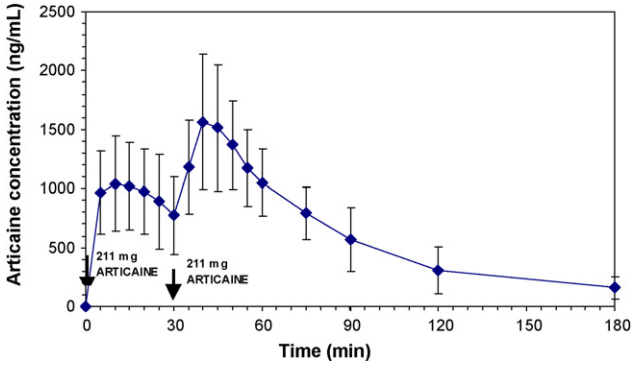

Fig. 4. Observed plasma concentration–time profile (mean ± S.D.) of articaine after a twofold submucosal infiltration injection of 211 mg (time of injection indicated by an arrow) articaine with epinephrine in six volunteers.

Under our experimental conditions, the carry-over effect was found to be minimal with carry-over rates lower than 0.2% of the LOQ.

3.2.3. Extraction recoveries

Articaine extraction recoveries from human plasma at three levels of concentration are summarized in Table 1. These results indicate that the single-step liquid–liquid extraction procedure used in this assay lead to a moderate but acceptable recovery (~40%). A preliminary evaluation of different extraction solvents such as dichloromethane, chloroform, hexane, ethyl-acetate or protein precipitation with methanol, acetonitrile and perchloric-acid showed that extraction with ether was the most suitable method. Although these extraction conditions appear to be less efficient than the solid-phase extraction method described by Richter and Oertel [9] (recovery from serum found to be 72%), our findings are reproducible at each concentration studied (R.S.D. < 7%; $n = 5$) and good calibration curves and coefficients of determination could be obtained to ensure a satisfactory and precise analysis of articaine in plasma for our purpose of a pharmacokinetic study.

3.2.4. Specificity and ion-suppression test

The analysis of 10 blank plasma samples from healthy volunteers showed no interfering peak on the chromatograms. In our assay, no significant ion-suppression effect has been observed with all blank plasma extracts at the expected retention times of the

articaine, and IS (Fig. 3). In agreement with Müller et al. [10], ion-suppression effects were detected at the LC front (retention time < 1.5 min, i.e. during the elution of non-retained compounds), but this did not really interfere with the ionisation of compounds assayed in positive ionisation mode. Other ion-suppression experiments have shown that no reciprocal ion suppression of articaine and trazodone occurred during their co-elution.

3.2.5. Method application

The mean concentration–time profiles for articaine in plasma obtained from six patients participating in an ongoing clinical study is shown in Fig. 4. Detailed pharmacokinetic data for all subjects ($n = 20$) enrolled in the clinical study will be reported in a separate article.

In conclusion, we have developed a LC–MS method to quantify articaine in human plasma. In addition to its specificity, this assay demonstrates acceptable precision and accuracy and has a short analysis run-time. This assay method has been successfully applied to a preliminary pharmacokinetic study following submucosal infiltration injection of the local anesthetic agent articaine hydrochloride in volunteers.

References

[1] S.F. Malamed, S. Gagnon, D. Leblanc, J. Am. Dent. Assoc. 132 (2001) 177–185.
[2] D. Isen, J. Dent. Qué. 25 (2001) 473–480.
[3] N. Nasseripour, T.T.H. Pham, C.D. Arreto, C.B. Wierzba, Actual OdontoStomatol. 210 (2000) 221–236.
[4] A. Van Der Auwera, A. Boukari, N. Meyer, S. Bahi, A. Feki, Med. Buc. Chir. Buc. 13 (2007) 187–198.
[5] R. Oertel, U. Ebert, R. Rahn, W. Kirch, Reg. Anesth. Pain Med. 24 (1999) 524–528.
[6] I.S. Holland, L.F.A. Stassen, Br. J. Oral Maxillofac. Surg. 34 (1996) 243–247.
[7] D.J. Edwards, M.R. Brickley, J. Horton, M.J. Edwards, J.P. Shepherd, Br. J. Oral Maxillofac. Surg. 36 (1998) 333–340.
[8] R. Oertel, K. Richter, K. Weile, Th. Gramatté, A. Berndt, K. Feller, Methods Find. Exp. Clin. Pharmacol. 15 (1993) 541–547.
[9] K. Richter, R. Oertel, J. Chromatogr. B 724 (1999) 109–115.
[10] C. Müller, P. Schäfer, M. Ströhrtzel, S. Vogt, W. Weinmann, J. Chromatrogr. B 773 (2002) 47–52.