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# Solid-phase extraction and high-performance liquid chromatographic determination of articaine and its metabolite articainic acid in human serum

Klaus Richter*, Reinhard Oertel

*Institut für Klinische Pharmakologie, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Fiedlerstraße 27, D-01307 Dresden, Germany*

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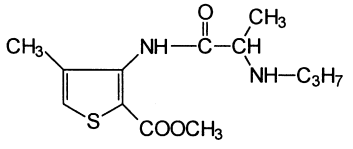
**Abstract**

A new method is described using solid-phase extraction (SPE) for preconcentration of articaine and the metabolite articainic acid and high-performance liquid chromatography (HPLC) for the determination of both compounds in human serum. Articaine and articainic acid were extracted in one step with SDB-RPS disk cartridges after precipitation of the serum proteins by perchloric acid. The HPLC separation was then performed on a reversed-phase $C_8$ column using phosphate buffer–acetonitrile (88:12, v/v). UV absorption at 274 nm was used for measuring the analytes with a low limit of quantitation of about 10 ng/ml, which is appropriate for pharmacokinetic studies of low dose submucosal injections of the local anaesthetic agent articaine hydrochloride in dentistry. © 1999 Elsevier Science B.V. All rights reserved.

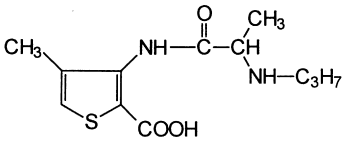
*Keywords:* Articaine; Articainic acid

## 1. Introduction

Articaine hydrochloride [4-methyl-3-(2-propylaminopropionamido)thiophene-2-carboxylic acid methyl ester hydrochloride] is a widely used local anaesthetic agent in dentistry [1]. The ester is quickly hydrolysed by serum esterases [1,2] to articainic acid (Fig. 1). Therefore, both the parent drug articaine and its metabolite articainic acid must be considered for the kinetic evaluation. A method for the determination of both, articaine and articainic acid, using high-performance liquid chromatography (HPLC) [3] was developed appropriate for inves-




Articaine




Articainic acid

Fig. 1. Structural formulae for the analytes.

*Corresponding author. Tel.: +49-351-458-2104, fax: +49-351-458-4341; e-mail: klaric@rcs.urz.tu-dresden.de

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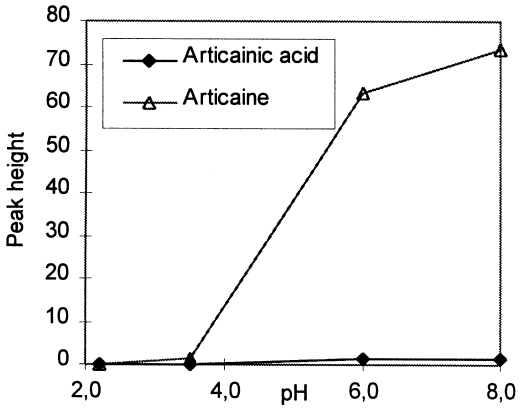


Fig. 2. Extraction recovery of the two compounds with methyl *tert*.-butyl ether from buffers of pH=2.2 and pH=8, respectively.

tigations on humans who had received submucosal injections of 80 mg articaine hydrochloride. After precipitation of the serum proteins by perchloric acid, 70 μl of the clear supernatant was direct injected for HPLC with a limit of quantitation of about 100 ng/ml. However, this method was not sensitive enough for studies using submucosal injections of only 16 mg articaine hydrochloride. Articaine, a lipophilic weak base ($pK_a$=7.8), may be extracted—dependent on the pH value—with methyl *tert*.-butyl ether, but not the more polar articainic acid, as shown in Fig. 2. Hence, solid-phase extraction (SPE) procedures with styrene–divinyl benzene polymer SPE cartridges were investigated for preconcentration of the two analytes from the supernatant of the protein precipitation. The new method leads to an improved sensitivity for articaine hydrochloride and articainic acid with a limit of quantitation of 10 ng/ml. Disk-cartridges were used due to their lower elution volume compared with non-disk-cartridges containing larger amounts of polymer beads.

## 2. Experimental

### *2.1. Chemicals*

Articaine hydrochloride and articainic acid were provided by Hoechst, Hoechst Marion Roussel Deutschland (Bad Soden am Taunus, Germany). Acetonitrile LiChrosolv (for chromatography), methanol LiChrosolv (for chromatography), methyl *tert*.-butyl ether LiChrosolv (for chromatography), and potassium dihydrogenphosphate (for molecular biology) were purchased from Merck (Darmstadt, Germany), and perchloric acid (70%), from Riedel-de Haen (Seelze, Germany). Pure water (18.2 MΩ) was obtained using the ion-exchange system RS 40E, SG Ionenaustauscher (Barsbüttel, Germany).

3M Empore high-performance extraction disk cartridges SDB-XC and SDB-RPS (4 mm/1 ml) were delivered by Varian (Darmstadt, Germany).

### *2.2. HPLC method*

The Gynkotek-HPLC system was set up with a M480Gmic pump, a GINA 50 T auto-injector, a column oven STH 585 and a diode array spectrophotometric detector UVD 340 S (Gynkotek, Germering, Germany). The chromatography data system Chromeleon (V 3.12, Gynkotek) was used for controlling the HPLC system, the diode array detector, evaluation of the chromatograms and data processing. A reversed-phase column RP-8, 5 μm particle size, 125 mm×3 mm I.D., cartridge CC 125/3 Nucleosil 50-5 endcapped (Macherey-Nagel, Düren, Germany) was used. The column was maintained at a temperature of 35°C. The mobile phase was a mixture of 880 ml potassium dihydrogenphosphate (0.02 mol/l 0.5 ml phosphoric acid, pH 3) and 120 ml acetonitrile. The solvent flow-rate was 1 ml/min.

### *2.3. SPE procedure*

An automated Gilson Sample Processor for SPE (ASPEC XL, ABIMED, Langenfeld, Germany) was used. The retention of articaine and articainic acid on styrene–divinyl benzene polymer (SDB-XC) and a sulfonated styrene–divinyl benzene polymer (SDB-RPS) disk-cartridges were investigated from solutions at pH values of 2.2 and 8. Both articaine and articainic acid were extracted from the solution with pH 2.2 using SDB-RPS, but at pH 8 articainic acid was not extracted completely. Vice versa, the two analytes were extracted with SDB-XC at pH 8 but not completely at pH 2.2. Furthermore, the analytes extracted under acidic conditions can be eluted from

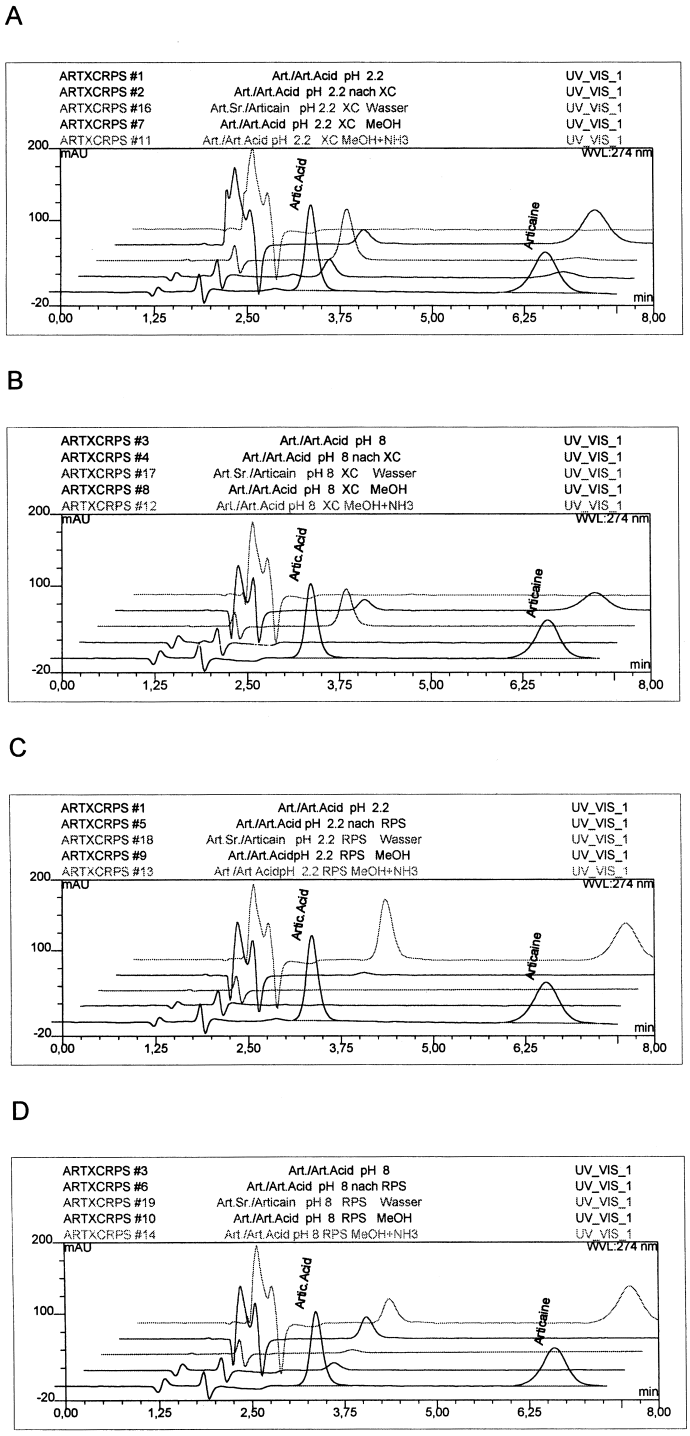


Fig. 3. SPE of articaine and articainic acid using SDB-XC (A, B) and SDB-RPS (C, D) at pH=2.2 and pH=8. Chromatograms (from bottom to top) of start solution; filtrate of the start solution; eluate with water; with methanol and with methanol+ammonia. The mobile phase used for these chromatograms contained 15% acetonitrile.

SDB-RPS only with methanol containing 1% of ammonia. The chromatograms in Fig. 3 demonstrate the surprising behavior of the two compounds. That is why we then only used SDB-RPS disk-cartridges for serum sample preparation. The following procedure was evaluated for the automated SPE of the analytes:

Condition: 0.5 ml methanol, 0.5 ml air, 0.5 ml water and 1 ml air. Load: 0.8 ml sample and 2 ml air. Wash: 0.8 ml phosphoric acid (0.5%) containing 20% of methanol, 1.5 ml air, 0.7 ml water and 2 ml air. Elution: 0.5 ml methanol containing 1% of ammonia and 1.2 ml air.

### *2.4. Sample preparation*

Standard samples for validation were prepared from 1 ml ice-cold blank serum given into 1.5-ml conical Eppendorf vessels containing the appropriate amounts of the analytes listed in Table 1. Then 50 μl of perchloric acid was added and mixed thoroughly (REAX 1 R, Heidolph, Kelkheim, Germany). After another 10 min, the samples were mixed again and centrifuged for 10 min at 16 000 *g* (Centrifuge 5415 C, Eppendorf-Netheler-Hinz, Hamburg, Germany); 840 μl of the clear supernatant was transferred into ASPEC sample tubes and 800 μl was taken for SPE. The eluates of the analytes with methanol containing 1% ammonia were evaporated to dryness at 70°C with a stream of air (Techne Sample Concentrator, Model SC-3, thermo-DUX, Wertheim, Germany). The residues were dissolved in 50 μl mobile phase for HPLC, transferred into conical autosampler vials and a 40-μl aliquot was then injected onto the column.

Serum samples (1 ml) from subjects were, immediately after thawing, assayed together with standard samples for the calibration.

### *2.5. Standard solutions*

Stock solutions of articaine hydrochloride and articainic acid were prepared by dissolving each of the substances in water to a final concentration of 1 mg/ml. Equal volumes of the solutions were mixed and working solutions were obtained by further dilution of this mixture with water.

## 3. Results

### *3.1. Chromatography*

The retention times of articainic acid and articaine were about 3.5 and 9.5 min, respectively, and overall chromatographic run time was 12 min. Typical chromatograms are shown in Fig. 4. The mobile

Table 1
Precision of the analytical method for articaine hydrochloride and articainic acid from six sets of samples on different days

| Concentration added (ng/ml) | Concentration found (mean±SD) (ng/ml) | Coefficient of variation (%) |
|---|---|---|
| *Articaine hydrochloride* | | |
| 10 | 9.3±1.0 | 10.4 |
| 20 | 19. 9±1.6 | 8.1 |
| 50 | 52.1±5.7 | 10.9 |
| 100 | 101.6±9.4 | 9.2 |
| 200 | 194.6±12.0 | 6.2 |
| 500 | 500.5±17.9 | 3.6 |
| 1000 | 983.9±30.2 | 3.1 |
| *Articainic acid* | | |
| 10 | 9.0±1.0 | 10.6 |
| 20 | 20.3±1.5 | 7.6 |
| 50 | 51.1±3.3 | 6.5 |
| 100 | 103.4±7.9 | 7.7 |
| 200 | 198.7±13.2 | 6.7 |
| 500 | 507.1±20.0 | 4.0 |
| 1000 | 972.2±35.6 | 3.7 |

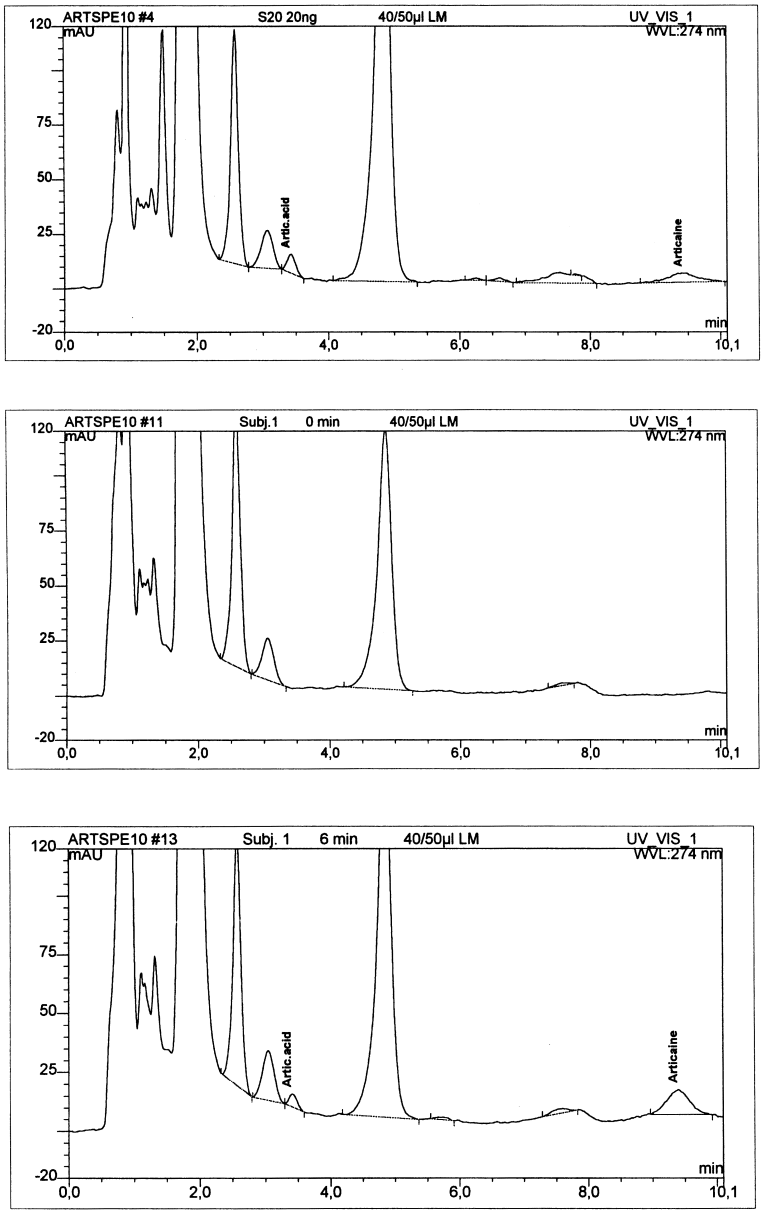


Fig. 4. Chromatograms of serum samples. S20=standard 20 ng/ml; 0 min=blank; 6 min=serum sample of a subject 6 min after an oral submucosal injection of 16 mg of articaine hydrochloride (articaine hydrochloride=55 ng/ml. articainic acid=14 ng/ml).

phase and column used in HPLC were selected for separating articainic acid from the preceding peak of an endogenous substance. This separation may be adjusted by slight changes in the acetonitrile content of the mobile phase. Pooled supernatant of protein precipitation is well suited for this adjustment and also for preparation of quality control samples.

### *3.2. Precision and linearity*

Precision and accuracy of the method were assessed by the determination of seven concentrations in six sets of independently prepared series of spiked serum samples, as shown in Table 1. These sets were prepared on different days. The lower limit of

quantitation, i.e., a coefficient of variation (C.V.)< 10% for six repeated measurements, is about 10 ng/ml for articaine hydrochloride and articainic acid. The linear regression (with weighting 1/conc.) shows linearity in the range of at least 10–1000 ng/ml for articaine hydrochloride and articainic acid ($r$>0.998).

### 3.3. Extraction recovery

The recovery of articaine and articainic acid was calculated from the peak heights of processed samples containing 40 and 400 ng/ml, respectively, compared to the peak heights of the direct injected amounts of the two analytes. The recovery from serum was found to be 72% for articaine hydrochloride and 78% for articainic acid, as shown in Table 2.

### 3.4. Stability

The half-life of articaine hydrolysed by esterase in serum was 3 h at 22°C and 16 h at 4°C, respectively [2]. We can confirm from our experience [1,3] that articaine is stable in serum stored at −20°C for at least three months and in the supernatant of the deproteinated serum for at least six months.

Table 2
Recovery of articaine hydrochloride and articainic acid, calculated from the reduced peak heights (=peak height/amount) of extracted serum samples and the reduced peak heights of direct injected amounts

| Injection | Amount injected (ng) | Articaine hydrochloride reduced height (mAU/ng) | Articainic acid reduced height (mAU/ng) |
|---|---|---|---|
| *Direct* | 40 | 0.408 | 1.108 |
| | 40 | 0.415 | 1.128 |
| | 40 | 0.425 | 1.101 |
| | 40 | 0.451 | 1.032 |
| | 400 | 0.411 | 1.067 |
| | 400 | 0.409 | 1.068 |
| | 400 | 0.402 | 1.054 |
| | 400 | 0.401 | 1.057 |
| | 400 | 0.436 | 1.120 |
| | 400 | 0.434 | 1.116 |
| Mean direct | | 0.419 | 1.085 |
| SD | | 0.017 | 0.033 |
| C.V. (%) | | 3.9 | 3.1 |
| *Extracted* | 40 | 0.337 | 0.867 |
| | 40 | 0.335 | 0.944 |
| | 40 | 0.289 | 0.847 |
| | 40 | 0.313 | 0.793 |
| | 40 | 0.257 | 0.770 |
| | 400 | 0.298 | 0.849 |
| | 400 | 0.293 | 0.830 |
| | 400 | 0.288 | 0.822 |
| | 400 | 0.303 | 0.866 |
| | 400 | 0.316 | 0.867 |
| Mean extracted | | 0.303 | 0.846 |
| SD | | 0.024 | 0.048 |
| C.V. (%) | | 7.9 | 5.6 |
| Recovery (%) | | 72.2 | 77.9 |

### *3.5. Testing of the method with real serum samples of a subject after a submucosal injection of 16 mg articaine hydrochloride.*

Fig. 5 shows the concentration–time curve of articaine hydrochloride and its metabolite articainic acid in a subject after an oral submucosal injection of 16 mg articaine hydrochloride. The sensitivity of the method is sufficient for determination of the analytes in human serum samples even in the case of low dose administration.

### *3.6. Comment on the solid-phase extraction of articaine and articainic acid*

The surprising retention behavior of articaine and articainic acid (Fig. 1) on styrene–divinyl benzene polymers (Section 2.3 and Fig. 3) was found by accident using these resins due to their pH stability. It was not to be expected that both analytes could be extracted both at pH 2.2 and at pH 8. But the hydrophobic interaction is stronger for articaine than for the more polar articainic acid. With the sulfonated resin articaine shows a very strong interaction not depending on the pH value, and the elution is only possible with methanol containing 2% ammonia solution. At pH 2.2, articainic acid in non-ionized form exhibits the same behavior as articaine; at pH 8, however, the interaction is lowered due to the ionized carboxylic group. The mechanism of the interaction between articaine and the sulfo group is not yet clear. If the sulfo group, in this case, would act as a cation exchanger, then articaine should be eluted with the strong acidic solution prepared from serum and perchloric acid.

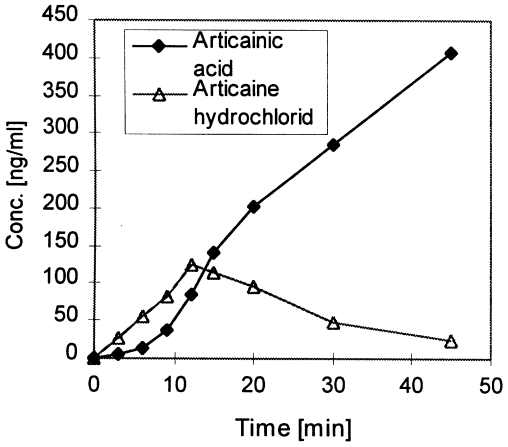


Fig. 5. Serum concentration–time curves of articaine hydrochloride and articainic acid in a subject after an oral submucosal injection of 16 mg articaine hydrochloride.

## 4. Conclusions

The preconcentration of articaine and articainic acid using SPE with sulfonated styrene–divinyl benzene polymer disk-cartridges in one step improves the sensitivity of the method up to about 10 ng/ml, appropriate for pharmacokinetic studies with submucosal injection of a low articaine hydrochloride dose on humans as shown in Fig. 5. The automated SPE gives an excellent reproducibility even without an internal standard, and a large number of samples can be processed daily.

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