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Articaine & Epinephrine Injection according to USP method

- **U.S. Pharmacopeia 37-NF32 (2014)**
- **Determination of the Epinephrine contents**
- **Analysis of organic and epinephrine-related impurities**
- **Reproducible & Robust**

Introduction

Articaine in combination with epinephrine is used as an anesthetic for dental procedures in a number of European countries, US and Canada. Like other local anesthetic drugs, articaine causes a transient and completely reversible state of anesthesia (loss of sensation). This drug was first synthesized by Rusching in 1969 and brought to the market in Germany by Hoechst AG under the brand name Ultracain [1]. It was approved by the FDA in April 2000 and became available two months later in the United States under the brand name Septocaine [2]. The U.S. Pharmacopoeia monograph for Articaine Hydrochloride and Epinephrine injections describes a method for the analysis of the Epinephrine contents and organic impurity analysis [3]. This method is based on HPLC in combination with electrochemical detection in the DC mode on a glassy carbon working electrode [4].

Summary

The Epinephrine analysis was evaluated on an Antec ALEXYS LC-EC analyzer, using the method and conditions described in the official 2014 USP monograph 37-NF32 for Articaine Hydrochloride and Epinephrine injection [3]. In this application note typical results obtained with the ALEXYS® system are reported, demonstrating its performance for the analysis of the Epinephrine contents, organic impurities and Epinephrine-related impurities in anesthetic products based on Articaine with Epinephrine.

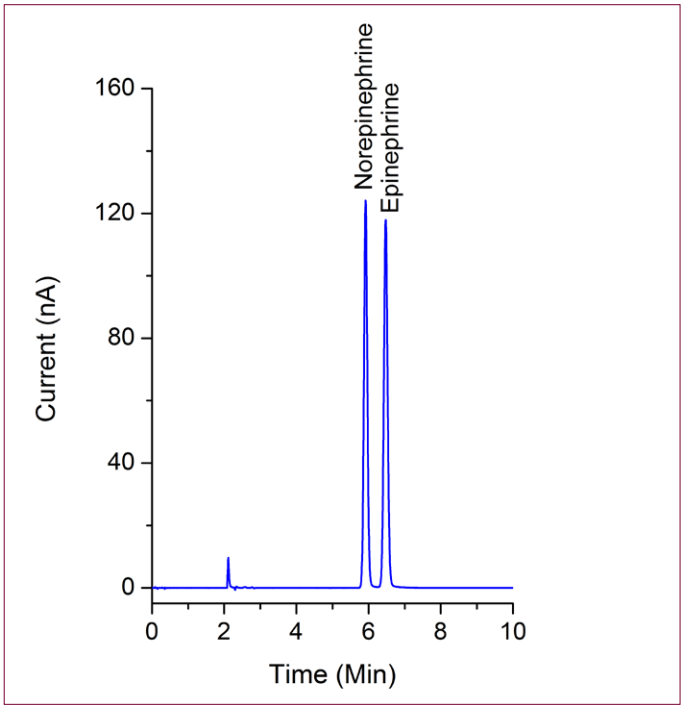

Figure 1: 2 µL injection of a 22 µg/mL Epinephrine Bitartrate RS and 20 µg/mL Norepinephrine Bitartrate RS solution in 0.5 mg/mL Potassium Metabisulfite (System suitability solution as described in the USP monograph).

Method and results

Separation

Separation of Epinephrine and its related impurities is achieved using a reverse phase C8 column in combination with an acidic buffered solution with 1-Heptanesulfonate as ion-pairing agent and methanol as organic modifier (isocratic elution).

In the monographs the use of the following column type is described for the separation of Epinephrine: size 250 mm, ID 4.0 mm, 5 µm packing L7. The USP packing L7 is described as: Octylsilane chemically bonded to totally porous or superficially porous silica particles 1.5 to 10 µm in diameter, or a monolithic rod. A Phenomenex Luna 5µ C8(2), 250 x 4.6 mm column was chosen for the method evaluation. This specific stationary phase is listed in the USP L7 packing list. Note that the ID of the column is slightly larger (4.6 mm), such variation in internal diameter is allowed by the USP [6].

Table 1

LC-EC Conditions	
HPLC	ALEXYS Epinephrine Analyzer.
Column	4.6 mm ID x 25 cm, 5µm, packing L7
Mobile phase	50 mL of glacial acetic acid and 930 mL water. Adjust with 2N sodium hydroxide to a pH of 3.4. In this solution, dissolve 1.2g of sodium 1-heptanesulfonate and add 1 mL of 0.1 M edetate disodium and 0.298g of potassium chloride. Add 150 mL methanol
Diluent	0.5 mg/mL potassium metabisulfite in water
Flow rate	1.0 mL/min
$V_{injection}$	2 µL
Temperature	30°C for separation and detection
Flow cell	Sencell™ with 2mm Glassy Carbon working electrode, Ag/AgCl (salt bridge) reference electrode and stainless steel auxiliary electrode, AST setting 2
Potential	E= +0.65 V
I-cell	ca. 3 nA
ADF	0.5 Hz
Range	1 µA and 1 nA (for LOD measurements)

Detection

For the detection of Epinephrine and its related impurities, am-perometric detection in Direct Current (DC) mode is mandatory using a Glassy Carbon (GC) working electrode and Ag/AgCl reference electrode. The Antec SenCell matches these requirements and was used in this evaluation. The cell was set to a static DC cell potential of +650 mV, the cell current was typical 3 nA under the measurement conditions listed in table 1. The temperature for separation and detection was 30°C. Note that for optimal temperature control of the electrochemical detector the ambient temperature in the laboratory does not exceed 23°C.

System Suitability

A chromatogram of an 2 µL injection of a 22 µg/mL Epinephrine Bitartrate RS and 20 µg/mL Norepinephrine Bitartrate RS solution in 0.5 mg/mL Potassium Metabisulfite is show in figure 1 (system suitability solution as described in the USP monograph). Besides the USP system suitability solution also solutions were analysed containing two known epinephrine-related substances, Adrenalone and Epinephrine sulfonic acid, see figure 2.

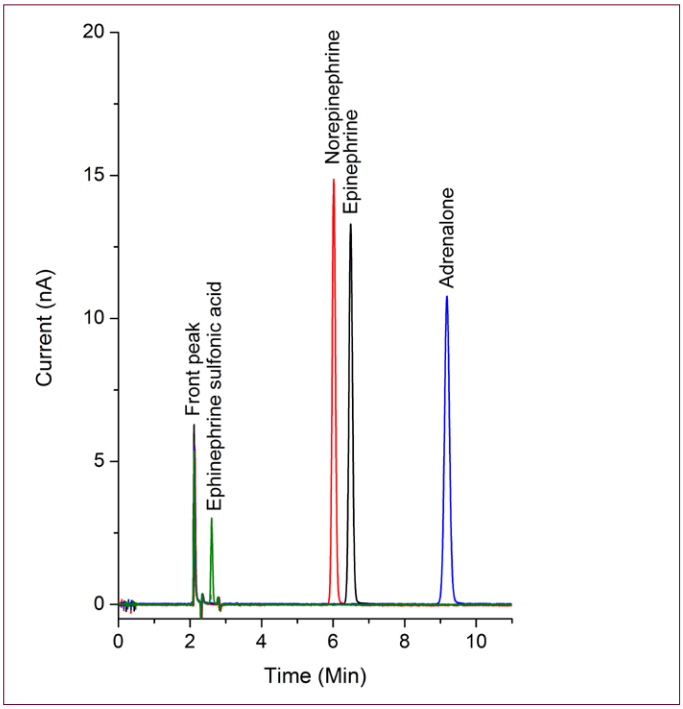

Figure 2: Identification of Epinephrine and related compounds: Chromatograms of 2 µL injections of 2.5 ppm (2.5 µg/mL) solutions of: (1) USP Epinephrine bitartrate RS (black), (2) USP Norepinephrine bitartrate RS (red), (3) Epinephrine sulfonic acid (green) and (4) Adrenalone HCl (blue) in 0.5 mg/mL Potassium metabisulfite.

The retention times of the substances are listed in table 2. The relative retention times for Epinephrine and Norepinephrine are in correspondence with the ones indicated in the USP monograph.

Table 2

Retention Time		
Component	*Retention time (min)*	*Relative Retention time (RRT)**
Epinephrine sulfonic acid	2.60	0.40
Norepinephrine	5.86	0.91
Epinephrine	6.47	1.0
Adrenalone	9.08	1.40

*) Relative retention time (RRT) with reference to Epinephrine (6.47 min).

It is evident from figure 2 that the response of Epinephrine Sulfonic Acid (ESA) is significantly lower than that of the other components. This is most likely due to the fact that the optimal oxidation potential for Epinephrine Sulfonic Acid is at a higher potential. The USP monograph demands a potential setting of E= +0.65 V for the analysis, which is not necessarily the most optimal potential for all compounds.

In the USP monograph for Articaine and Epinephrine the following system suitability requirements are specified:

- Resolution: not less than 1.5 between the Norepinephrine and Epinephrine peak.
- Tailing factor: not more than 2.0 for the Epinephrine peak.
- Relative standard deviation: not more than 1% for the Epinephrine peak from 6 injections (n=6).

The system suitability is evaluated using the chromatograms obtained with the standard solution of 22 µg/mL Epinephrine Bitartrate RS and 20 µg/mL Norepinephrine Bitartrate RS solution in 0.5 mg/mL Potassium Metabisulfite (system suitability solution). The results are listed in table 3, it is evident that the system suitability requirements are met for all performance parameters.

Table 3

USP System Suitability Requirement		
Parameter	*USP criteria*	*Measured*
Resolution between Norepinephrine and Epinephrine	> 1.5	2.9
Tailing factor (Epinephrine)	< 2.0	1.1
RDS n=6 (Peak area Epinephrine)	< 1%	0.4

Linearity, repeatability and LOD

The linearity of Epinephrine and Norepinephrine were investigated in the concentration range of 4 – 22 µg/mL (20 µg/mL for Norepinephrine). For both components the correlation coefficients were better than 0.999 for peak areas. The relative standard deviation (% RSD) in peak area was determined for 6 replicate injections of the system suitability solution. The RSDs in peak area were 0.4% for both components.

A 2.5 ppb (2.5 ng/mL) standard mix of Epinephrine, Norepinephrine, Adrenalone and Epinephrine sulfonic acid was injected to assess the Limit Of Detection (LOD) of the compounds. See figure 3. The calculated concentration LODs are listed in table 4. The LOD here is based on a 2 µL injection and defined as the concentration that gives a signal that is three times the peak-to-peak noise.

Table 4

LOD		
Component	*LOD (nM)*	*LOD (ppb)*
Epinephrine sulfonic acid	14.4	3.8
Norepinephrine	2.4	0.8
Epinephrine	2.4	0.8
Adrenalone	4.1	0.9

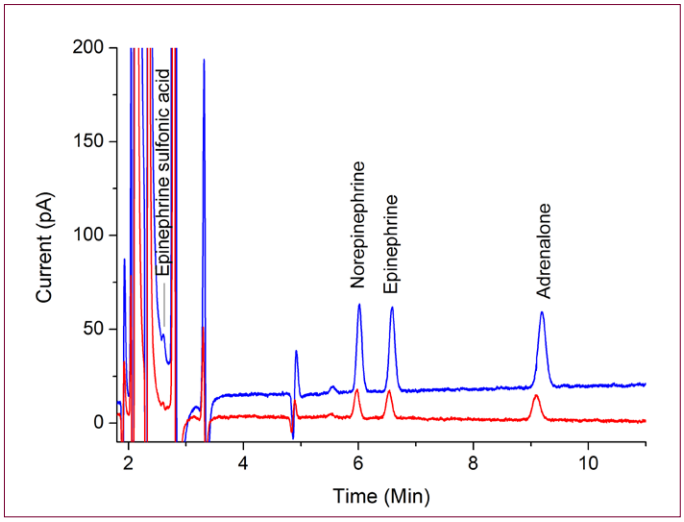

Figure 3: Chromatograms of a 2.5 ppb standard mix of epinephrine and related compounds. Injection volume 2µL (red curve) and 10 µL (blue curve).

As can be seen from table 4 and figure 3 all compounds have a detection limit below 1 ppb, with the exception of epinephrine sulfonic acid. The USP monograph demands an injection volume of 2 µL. However by increasing the injection volume to for example 10 µL the concentration LOD can be improved effectively by more than a factor of 3 as demonstrated in figure 3 when required. Under the USP conditions the Limit of Quantitation is approximately 2.5 ppb (except Epinephrine sulfonic acid).

Sample Analysis

To evaluate the Epinephrine assay and Epinephrinerelated impurity analysis described in the USP monograph, two stressed Epinephrine samples in metabisulfite were analyzed. One sample was kept at acidic pH the other at mild alkaline conditions:

(1) 0.01% (100 ppm) epinephrine sample, acidic (pH 4)
(2) 0.01% (100 ppm) epinephrine sample, basic (pH 8-9)

Epinephrine assay

To determine the actual content of Epinephrine in the samples, 2µl of a 40x dilution of both 0.01% Epinephrine samples were injected. Based on the declared contents of 100 ppm, this corresponds to a final concentration of 2.5 ppm (2.5 µg/mL Epinephrine). The chromatograms of both diluted samples are shown in figure 4.

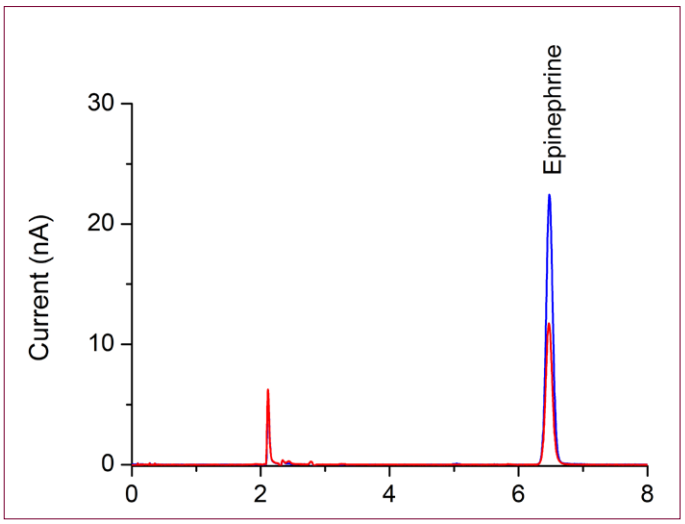

Figure 4: Chromatogram of (1) Blue curve: 0.01% Epinephrine sample pH 4, diluted 40x (2.5 ppm). Red curve: 0.01% Epinephrine sample pH 8-9, diluted 40x (2.5 ppm). Injection volume 2µL

The actual content of epinephrine in both samples was calculated using the response of a standard solution of 2.5 µg/mL Epineph-rine bitartrate RS in diluent, using the following calculation:

Result= (Ru/Rs) x (Cs/Cu) x 100%

Where:

Ru = Epinephrine peak area from the of sample solution
Rs = Epinephrine peak area from the of standard solution
Cs = Concentration of Epinephrine in the std. solution (mmol/L)
Cu = Nominal Concentration of Epinephrine in the sample solution (mmol/L)

Due to the fact that the standard and sample solutions originate from Epinephrine bitartrate (M = 333.29 g/mol) and Epinephrine base (M = 183.21 g/mol), respectively, it was necessary to correct for the molar mass. So instead of the concentration in mg/mL the molar concentration was used in the calculation. The calculated actual contents (%) of epinephrine in the stressed samples are listed in table 5 below.

Table 5

Epinephrine content		
Sample	*USP criteria (%)*	*Measured (%)*
0.01% Epinephrine, pH 4	90.0 – 115.0	90.6
0.01% Epinephrine, pH 8-9	90.0 – 115.0	48.1

It is evident that the more instable basic sample (pH 8-9), has a significant lower contents of Epinephrine (almost half less) than the acid sample, due to oxidation/degradation of Epinephrine.

Organic impurities, Limit of Epinephrine Related Compounds

To determine the contents of organic impurities and epinephrine related compounds, 2µL undiluted sample solution was injected and analyzed. The chromatograms of the undiluted acidic and basic 0.01% Epinephrine samples are shown in figure 5 and 6, respectively. The figures show a zoom-in on the baseline to visualize the impurities present in the samples. In the top-right corners of the figures the full chromatograms are shown.

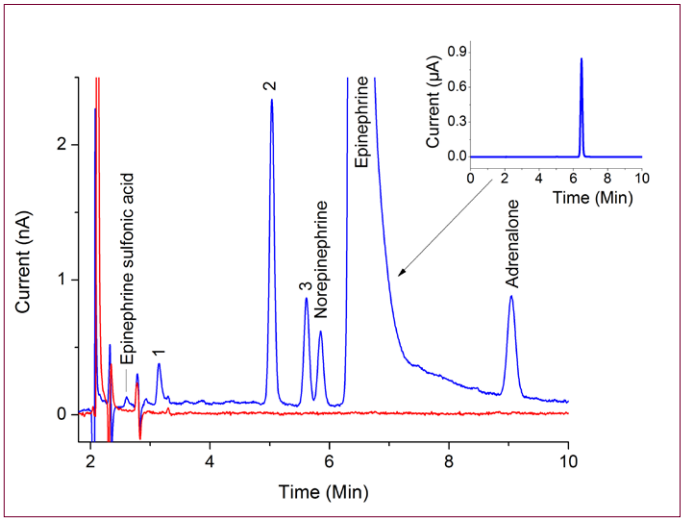

Figure 5: Blue curve: zoom-in on baseline of the chromatogram of the undilut-ed 0.01% Epinephrine sample pH 4. Top-right insert: full chromatogram. Red curve: blank injection of diluent. Injection volume 2 µL.

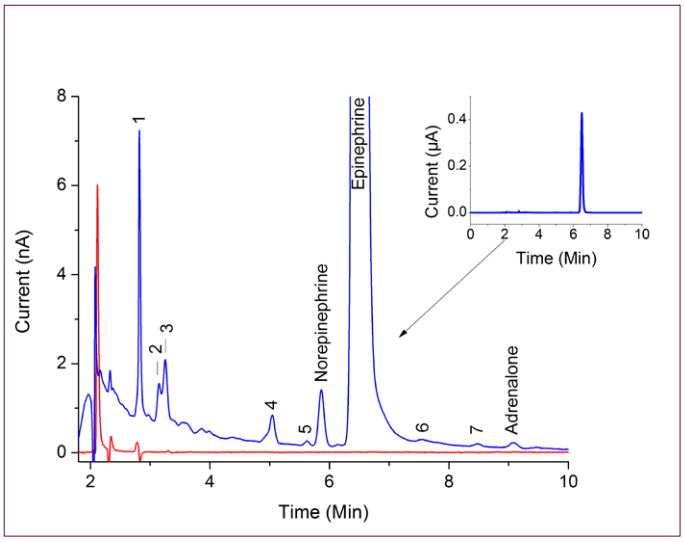

Figure 6: Blue curve: zoom-in on baseline of the chromatogram of the undilut-ed 0.01% Epinephrine sample pH 8-9. Top-right insert: full chromatogram. Red curve: blank injection of diluent. Injection volume 2 µL.

It is evident from figure 5 that besides the epinephrine related compounds there are 3 more unknown impurities with a significant response which can be quantified in the acidic sample. The blank injection shows that the peak next to Epinephrine sulfonic acid (right side) is a system peak and not a relevant impurity. The calculated contents (%) of impurities and epinephrine related compounds in the acidic Epinephrine sample are listed in table 6 below. The calculation used is the same as described in the sec-tion above for the determination of the content of epinephrine in the samples (USP Epinephrine assay). The response of the 2.5 ppm USP epinephrine standard was used for the calculation of the percentage of the impurities.

The USP acceptance criteria for the amount of impurities are:

- *Epinephrine sulfonate:* not more than 7.5% (Relative retention time approximately 0.46).
- *Specified impurity*: not more than 8% (Relative retention time approximately 0.52).
- *Any other individual impurity*: not more than 1%.
- *Total impurities*: not more than 10%.

Table 6

0.01% Epinephrine sample pH 4, Impurity analysis, Limit of Epinephrine related compounds			
Impurity	*RRT**	*Measured (%)*	*USP criteria (%)*
Epinephrine sulfonate**	0.40	0.003	7.5
Unknown 1	0.49	0.02	1
Unknown 2	0.78	0.19	1
Unknown 3	0.87	0.07	1
Norepinephrine	0.90	0.05	1
Adrenalone	1.40	0.11	1
Total		0.45	10

*) Relative retention time (RRT) with reference to Epinephrine (6.47 min). **) Epinephrine sulfonic acid.

Note that the relative retention time of Epinephrine sulfonic acid, 0.40 is slightly lower as indicated in the USP monograph (0.46). The contents of Epinephrine sulfonic acid was < 0.005%, well below the specified limit of 7.5%. All other quantified unknown impurities, as well as the total amount are below the specified limits and within the USP acceptance criteria.

The calculated contents (%) of impurities and epinephrine related compounds in the alkaline Epinephrine sample are listed in table 7. In this sample no detectable level of Epinephrine sulfonate is present. At a relative retention time of 0.44 a significant impurity peak is present. It is assumed (based on its relative position to Epinephrine sulfonate) that the peak at this retention time corresponds to the 'specified impurity' as mentioned in the USP monograph.

All quantified impurities or related compounds are below 1%, which is within the USP acceptance criteria. The total amount of quantified impurities was 0.58%, well within the USP limit of 10%.

Table7

0.01% Epinephrine sample pH 8-9, Impurity analysis, Limit of Epinephrine related compounds			
Impurity	*RRT**	*Measured (%)*	*USP criteria (%)*
Unknown 1**	0.44	0.21	8
Unknown 2	0.49	0.05	1
Unknown 3	0.50	0.09	1
Unknown 4	0.78	0.07	1
Unknown 5	0.87	0.01	1
Norepinephrine	0.91	0.12	1
Unknown 6	1.16	0.01	1
Unknown 7	1.31	0.01	1
Adrenalone	1.40	0.02	1
Total		0.59	10

*) Relative retention time (RRT) with reference to Epinephrine (6.47 min). **) It is assumed (based on its relative position to Epinephrine sulfonate) that the peak at this retention time corresponds to the 'specified impurity' as mentioned in the USP mono-graph.

Conclusion

The ALEXYS Epinephrine Analyzer provides a suitable solution based on the official method of the USP for the analysis of the composition and Epinephrine-related impurities in commercial Articaine with Epinephrine injectable anesthetics.

References

1. S.F. Malamed, *Handbook of local anaesthesia,* St. Louis, Mosby, 5ed, (2004), 71
2. FDA website: *http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/022010_septocaine_toc.cfm*
3. Articaine Hydrochloride & Epinephrine injection, *United States Pharmacopoeia (USP),* USP37-NF32 (2014), 1834 – 1836
4. R.J. Flanagan, D. Perret, R. Whelton, *Electrochemical detection in HPLC: Analysis of Drugs and Poisons,* Royal Society of Chemistry, 1ed, (2005)
5. <621> Chromatography general chapter, *United States Pharmacopoeia (USP),* USP37-NF32 (2014), 6376 – 6386

Ordering number

180.0055C	ALEXYS Epinephrine analyzer, including column & flow cell
250.1070B	Phenomenex Luna 5µ C8(2), 250 x 4.6 mm column

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.