A Sensitive Method for Direct Analysis of Impurities in Apramycin and Other Aminoglycoside Antibiotics Using Charged Aerosol Detection

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Overview

Purpose: To develop a sensitive non-derivatization method for impurity assessment of apramycin sulfate and other aminoglycoside antibiotics.

Methods: A 30min gradient method using hydrophilic interaction liquid chromatography with charged aerosol detection (HILIC-CAD) was developed for direct analysis of apramycin sulfate . Samples were pretreated with solid phase extraction (SPE) to remove sulfate ion for more accurate determination of impurities. The same sample was also analyzed with the SCX-UV method recommended by British pharmacopoeia (veterinary) 2013, which requires post column derivatization.

Results: 16 impurities of apramycin were detected at S/N ≥ 3 with the HILIC-CAD method. The SCX-UV method recommended by British Pharmacopoeia only detected just seven impurities. The HILIC-CAD method is much more sensitive than ELSD. With 20 µg apramycin sulfate on column, 7 impurities were detected by CAD at S/N > 3, while only 3 impurities were detected by ELSD. This method, with or without slight modification, was also used for impurity measurement of an additional eleven aminoglycoside antibiotics, including neomycin, gentamicin, kanamycin, streptomycin, tobramycin, amikacin, etimicin, netilmicin, sisomicin, ribostamycin and paromomycin.

Introduction

Aminoglycosides are a group of structurally similar antibiotics used to treat infections caused by aerobic gram-negative bacteria[1]. Analytical methods are required for rapid assessment of drug purity and detection of minor degredants. As they lack a strong chromophore, these compounds are not amenable to UV detection.

Apramycin is an antibiotic used in veterinary medicine. Reported methods for apramycin and impurities analysis usually involves pre- or post column derivatization followed by UV detection[2,3]. Such approaches are tedious and time consuming, and may not be able to detect all impurities.

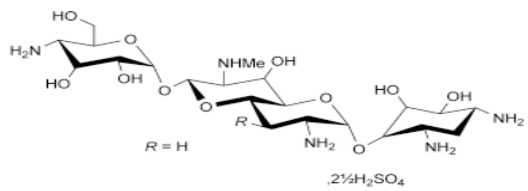
Aminoglycosides can be measured directly by charged aerosol detection without derivatization. Corona Veo is a universal mass-sensitive detector, Its response is independent of chemical structure, and does not require the presence of chromophores. Capable of measuring any nonvolatile and many semi-volatile analytes, charged aerosol detection enables accurate degradation studies and improved assessments of product purity. The Corona Veo is much more sensitive than other universal detectors like ELSD and RI, offering low nanogram quantitation. This poster presents a sensitive HILIC-CAD method for direct analysis of apramycin and other aminoglycosides. Method performance was compared to the British Pharmacopoeia HPLC-UV method and ELSD detection.

Methods

Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system with:

- Pump: LPG-3400SD
- Auto Sampler: WPS-3000TSL
- Column Compartment :TCC-3000RS
- Diode Array Detector: DAD-3000RS
- Charged aerasol detector: Corona Veo RS
- Varian ELSD 385-LC

FIGURE 1. Structure of Apramycin



Sample pre-treatment with SPE

SPE Column:	Dionex OnGuard II A
Sample solvent:	80% 5mM ammonium formate, 20% acetonitrile
Sample:	220.6 mg/mL in 2 mL sample solvent
SPE procedure:	Condition the SPE cartridge with 6 mL sample solvent, then pass the sample solution through the cartridge and wash the cartridge with additional 2 mL sample solvent . Combine collected loading eluent and wash solution for analysis. The final concentration of apramycin sample was 49 mg/mL. A 2 mL volume of sample solvent was treated with the same procedure and used as blank.

HILIC-CAD/ELSD method

Column:	ACCHROM (Beijing, China), Click XIon, 4.6 x 150mm, 5µm
Temperature:	30 ºC
Flow rate:	1 mL/min
Mobile Phase A:	Acetonitrile
Mobile Phase B:	500 mM ammonium formate, pH 2.9
Mobile phase C:	Water
Gradient	0 min., 70 %A, 20%B, 10% C 30 min., 21% A, 20%B, 59% C
Injection volume:	1 µL
Corona Veo RS:	55 °C evaporation temp., PFV 1.00, data rate 10 Hz, filter 5 s,
ELSD:	Nebulizer temp. 50 °C , evaporation temp. 70 °C

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SCX-UV method

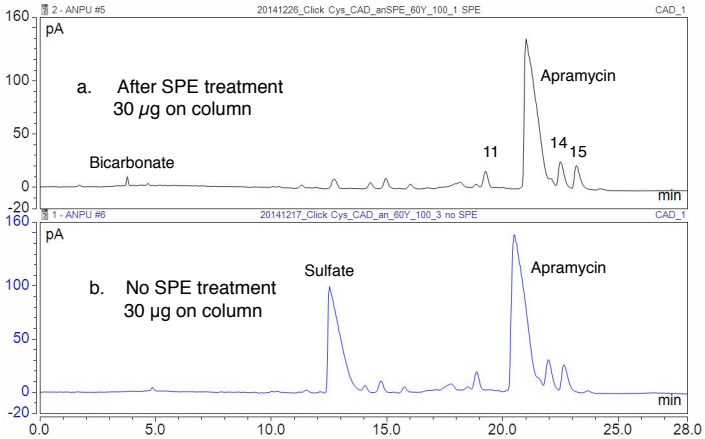
Column:	Venusil SCX-F 4.6 x 150 mm, 5 μm
Temperature:	30 ºC
UV Detector:	568 nm
Sample:	0.28 mg/mL
Injection volume:	20 µL

Chromatographic condition and post-column derivatization procedure are same as in British Veterinary Pharmacopoeia 2013

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 7.2

FIGURE 2. Effect of SPE Pre-treatment of Apramycin Sulfate Sample.



Results

Sample pre-treatment with SPE

Sulfate is a major interference for apramycin impurity assessment with a HILIC method. Without sample cleanup, some early eluting impurities were found to be masked under the huge sulfate peak and could not be detected. A Dionex anion exchange SPE cartridge On Guard II A was used to remove sulfate. Sulfate was retained on the SPE cartridge while the apramycin and impurities passed through the cartridge and collected for further analysis. Sulfate was replaced by bicarbonate after SPE, which has little interference with apramycin analysis, since it is volatile and elutes earlier than the peaks of interest. As seen in Figure 2, after removing sulfate, more impurities can now be detected. Recovery of three impurity peaks, labeled as peak 11, 14 and 15 in Figure 3a, was calculated to be 107%, 92% and 93%, respectively.

Comparison of HILIC-CAD method with SCX-UV method

The HILIC-CAD method was compared to the SCX-UV method recommended by British Pharmacopoeia (veterinary) 2013 version (BP2013) . As shown in Figure 3, the number of impurity peaks resolved and detected was greatly increased with the HILIC-CAD approach. About 16 impurity peaks were detected with the HILIC-CAD method at S/N > 3 (Figure 3A). The SCX-UV method only detected seven impurities, as not all of them could be derivatized by the SCX-UV approach (Figure 3B). Furthermore, the improved chromatographic resolution and peak shape allows for a higher sample load with the HILIC-CAD method enabling detection of low level impurities.

The effect of mobile buffer strength and pH on separation and peak shape was investigated. The method was optimized with 100mM ammonium formate at pH 2.9. The sample load was increased to 48.9 µg for the HILIC-CAD method and still maintains good peak shape with half peak width $W_{0.5}$ = 0.77min. While further increase of sample loading amount with the SCX-UV method caused significant peak broadening and results in decreased resolution between apramycin and impurity peaks.

FIGURE 3A. Impurity Analysis of Apramycin with the HILIC-CAD Method.

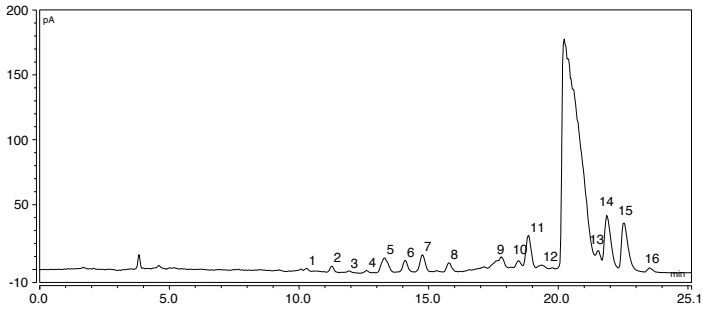
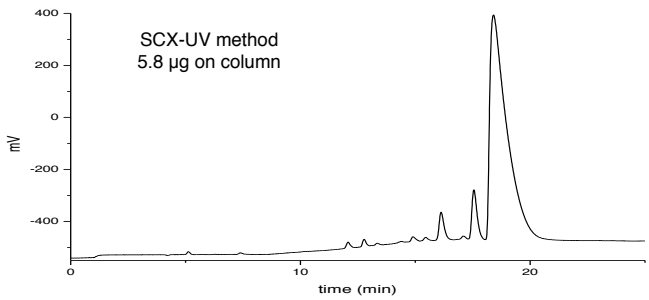


FIGURE 3B. Impurity Analysis of Apramycin with the SCX-UV Method.



Comparison of CAD and ELSD Detection

CAD and ELSD are both nebulization-based universal detection technologies. Comparison between CAD and ELSD under the same chromatographic conditions demonstrated that CAD is much more sensitive than ELSD. As shown in the chromatograms in Figure 4 and data summarized in Table 1, 16 impurities (S/N >3) were detected with CAD at an injected amount of 49.6 µg apramycin sulfate on column, while only 12 impurities were detected with ELSD at this level. When injection amount decreased to 20 µg on column, 7 impurity peaks were detected with CAD at S/N > 3, while only 3 peaks were detected with ELSD with much lower S/N compared to CAD.

The rapid decrease in analyte response at lower concentration found with ELSD is due to the sigmoidal nature of its response curve, resulting in much lower sensitivity.

FIGURE 4. Comparison of Detection Sensitivity Between CAD and ELSD.

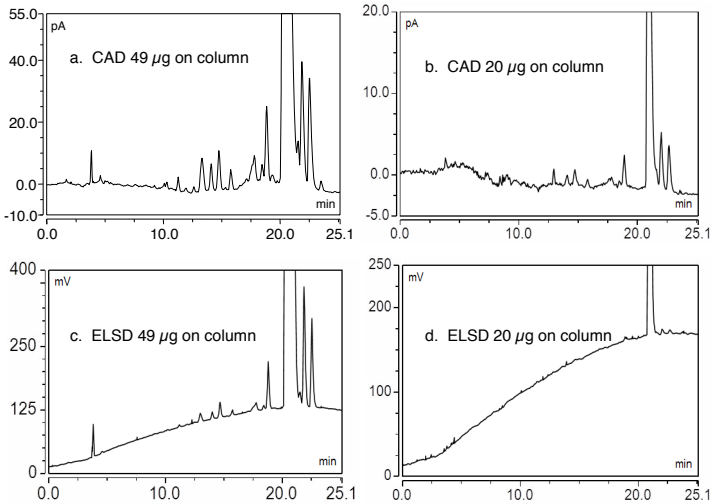


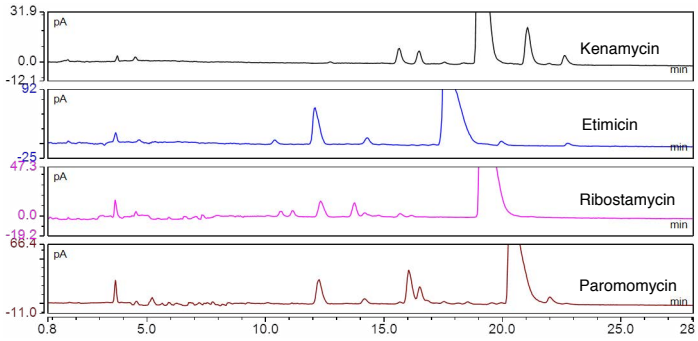
Table 1. Comparison of Detection Sensitivity Between CAD and ELSD

Peak Number	Retention Time (min)	S/N			
		49 µg on column		20 µg on column	
		CAD	ELSD	CAD	ELSD
1	10.30	4.9	6.4		-
2	11.27	10.4	8.8		-
3	11.94	3.0	-		-
4	12.61	4.2	-	6.4	-
5	13.32	24.4	19.4		-
6	14.10	19.6	19.5	3.9	-
7	14.76	29.0	44.6	6.2	-
8	15.78	15.0	14.0	3.2	-
9	17.79	18.6	22.9		-
10	18.47	10.1	12.3	-	-
11	18.85	51.7	137.9	11.8	3.7
12	19.34	4.8	-		-
13	21.55	12.7	19.3		-
14	21.87	79.5	293.9	21.3	5.6
15	22.52	77.0	238.2	17.9	4.1
16	23.52	6.9	-	-	-

Analysis of Other Aminoglycoside Antibiotics

This method has also been applied to impurity analysis of an additional eleven aminoglycoside antibiotics, including neomycin, gentamicin, kanamycin, streptomycin, tobramycin, amikacin, etimicin, netilmicin, sisomicin, ribostamycin and paromomycin. Figure 5 shows impurity analysis of kenamycin, etimicin, ribostamycin and paromomycin using the HILIC-CAD method. For some other aminoglycoside antibiotics, modification of the gradient may be required for optimized separation and resolution.

FIGURE 5. Chromatograms for impurity analysis of kenamycin, etimicin, ribostamycin and paromomycin using the HILIC-CAD method.



Conclusions

- The described HILIC-CAD method for apramycin enables more accurate impurity assessment, due to the universal detection of CAD and improved sample loading capacity. More than 16 impurities were detected. The SCX-UV method recommended by British Pharmacopoeia only detected seven impurities.
- Comparison between Corona Veo and ELSD detection showed that CAD is much more sensitive than ELSD. With 20µg sample on column, 7 impurities were detected at S/N ≥ 3 with CAD, while only 3 peeks at S/N ≥ 3 were detected with ELSD.
- Sample pretreatment with anion exchange SPE removes interference of sulfate ion and allows for more accurate determination of impurities.
- This method can also be used for the analysis of many other aminoglycoside antibiotics.

References

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