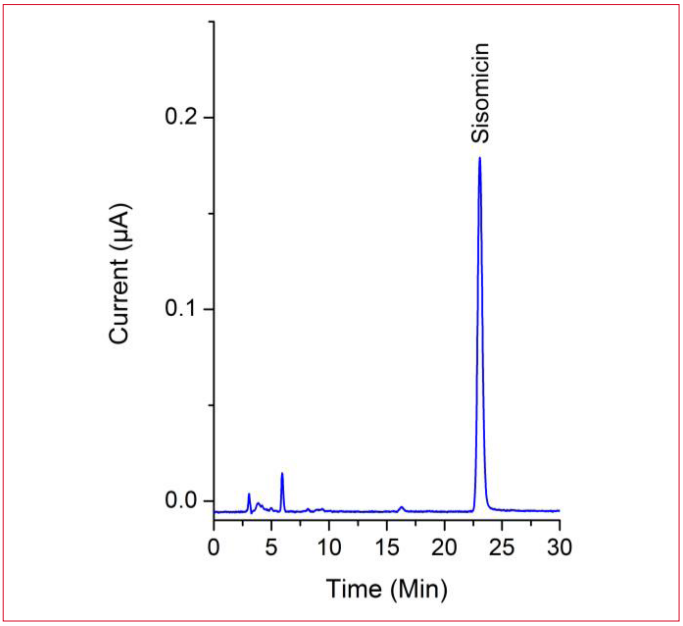

Figure 4: 20 µL injection 10 µg/mL Sisomicin sulphate CRS in mobile phase.

The system suitability was evaluated using the chromatograms of reference solution (c) and (d), see figure 3 and 2 respectively.

Table 3

EP system suitability Requirement

Parameter	*EP criteria*	*Measured*
Resolution between Impurity A & C1a	> 1.2	3.2
Resolution between C2 and C2b*	> 1.5	3.4
Signal-to-Noise ratio (Impurity A)	> 20	323

**) USP requirement: resolution between C2 and C2.*

The system suitability requirements are met for all parameters (table 3). Note: in the USP monograph the only system requirement is that the resolution between C2 and C2b is met (> 1.5).

Linearity and repeatability

The linearity of gentamicin was investigated in the concentration range of 25 – 200 µg/mL. For all gentamicin derivatives the correlation coefficients were better than 0.997 for peak areas. The relative standard deviation (RSD) in peak area for a triplicate injection of test solution (b) was ranging between 0.3 – 0.6% for C1, C1a, C2 and C2a. Only for C2b, with its relatively low peak height, the RSD was slightly higher (1.1%). The LOD (S/N ratio of 3) for Impurity A was 9 ng/mL.

Sample analysis

For a commercial sample the composition and related substances were analyzed and evaluated using the EP and USP acceptance criteria. The relative percentage of each gentamicin derivative in the commercial formulation was calculated using the peak area obtained from the chromatogram of test solution (b) shown in figure 1. The sum of all peak areas (C1a, C2 C2a, C2b and C1) corresponds to 100%.

Note that the calculation of the composition for the EP and USP slightly differ. In the EP the sum of C2, C2a and C2b is used; in the USP monograph the sum of C2 + C2a and the sum of C2b + C1. The results are shown in table 4; it is evident that the evaluated commercial sample met the acceptance criteria of both the EP and USP.

Table 4

EP system suitability requirement

Peak	*EP**		*USP**	
	Limits (%)	Calculated (%)	Limits (%)	Calculated (%)
C1a	10-30	28	10-35	28
C2	35-55	41	25-55	38
C2a				
C2b			25-50	34
C1	25-45	31		

**) The calculation of the composition for EP and USP slightly differ. In the EP the sum of C2, C2a and C2b is used; in the USP monograph the sum of C2 + C2a and the sum of C2b + C1.*

In addition, the EP monograph also describes acceptance criteria for impurity levels in commercial samples. For that purpose all impurities are quantified and compared to the response of the principal peak (Impurity A) obtained from the chromatogram of reference solution (c).

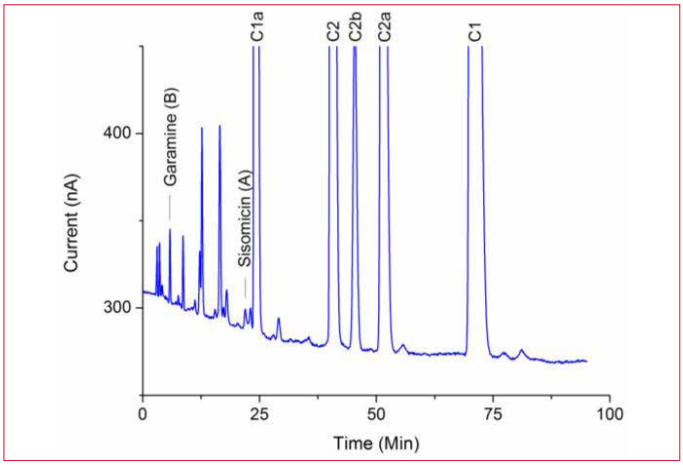

Figure 5: 20 µL injection of test solution (a) for the impurity quantification (1 mg/mLGentamicin sample in mobile phase).

The relative peak areas of all impurities in the commercial sample are listed in table 5.

Table 5

Impurity analysis		
Impurity	*RT (min)*	*Relative Peak Area**
1	3.1	0.07
2	3.6	0.07
3	4.2	0.02
Garamine (Impurity B)	5.8	0.11
5	7.6	0.02
6	8.7	0.11
7	11.2	0.02
8	12.2	0.13
9	12.7	0.39
10	15.5	0.03
11	16.5	0.50
12	17.3	0.04
13	18.0	0.11
Sisomicin (Impurity A)	22.0	0.08
15	23.1	0.08
17	28.0	0.02
18	29.1	0.09
22	55.6	0.07
24	77.2	0.07
25	81.1	0.11
Total	-	2.11

**) Relative Peak Area of the impurities are calculated in the following way: Relative peak area = Area of the impurity divided by the peak area of the principle peak in the chromatogram obtained with reference solution (c).*

The EP acceptance criteria for the amount of impurities are:

- *Impurity A, B (and any other impurity)*: Not more than 3x the peak area of sisomicin peak in the chromatogram of reference solution (c).
- *Total impurities:* Not more than 10x the peak area of sisomicin peak in the chromatogram of reference solution (c).
- *Discard limit:* Impurities with peak areas smaller than 0.5x the peak area of sisomicin peak in the chromatogram of reference solution (c) can be discarded.

The commercial sample met all impurity acceptance criteria. In fact the response of the majority of all impurities in the sample was under the discard limit of 0.5.

Table 6

Test and reference solutions EP	
Sample solution (a)	1 mg/mL Gentamicin sample in MP
Sample Solution (b)	0.2 mg/mL Gentamicin sample in MP
Reference Solution (a)	0.2 mg/mL Gentamicin for peak identification CRS in MP
Reference Solution (b)	1 mg/mL Sisomicin CRS in MP
Reference Solution (c)	10 µg/mL Sisomicin CRS in MP
Reference Solution (d)	20 µg/mL Sisomicin CRS with 100 µg/mL Gentamicin sample in MP

Conclusion

The ALEXYS Aminoglycosides Analyzer provides a reliable solution for the analysis of the composition & impurities in commercial Gentamicin Preparations following the official methods of the EP and USP.

Table 7

Reagents and standards	
NaOH 50%, carbonate-free	Boom Chemicals, pn 80011912
Trifluoroacetic acid, HPLC grade	Fischer Scientific, pn T/3258/PB05
Pentafluorpropionic acid, 97%	Acros Chemicals, pn 416920500
Acetonitrile, HPLC grade	Acros Chemicals, pn 268270025
Deionized Water. >18 MΩ-cm	Barnstead, Easy pure II
Gentamicin sulfate CRS, 16500 IU/vial	EP, pn G0200000, batch 8.1
Gentamicin for peak identification CRS*	EP, pn Y0001363, batch 1.0
Sisomicin sulphate CRS, 77.7%	EP, pn S0660000, batch 2.1

*)Gentamicin for peak identification CRS; not injected, reference chroma to- gram for peak identification downloaded from the following location: http://crs.pheur.org/db/4DCGI/View=Y0001363

Ordering information

180.0056W	Aminoglycoside Analyzer including Flowcell
250.1070B	ALA-525 column, 250x4.6mm, 5um C8

References

1. W.R. LaCourse, *"Pulsed Electrochemical Detection in High Performance Liquid Chromatography"*, John Wiley & Sons, New York, 1ed,1997.
2. Gentamicin sulphate, *European Pharmacopoeia (EP)*, 8.1, (2014) 2326 -2382
3. Gentamicin sulphate, *United States Pharmacopoeia* (USP), USP37-NF32, 3138-3139
4. V. Manyanga, K. Kreft, B. Divjak, J. Hoogmartens, E. Adams, *J. Chromatogr. A,* 1189, 347-354 (2008).
5. *Gentamicin Sulphate in pharmaceutical formulations,* Antec application note, 217_013

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