System suitability

In the EP monographs for Tobramycin the following system suitability requirement are specified:

- *Resolution:* minimum 3.0 between Kanamycin B (impurity A) and Tobramycin in the chromatogram obtained with reference solution (d), see figure 2.
- *Signal-to-Noise ratio:* minimum 10 for the principal peak in the chromatogram obtained with reference solution (b), see figure 3.

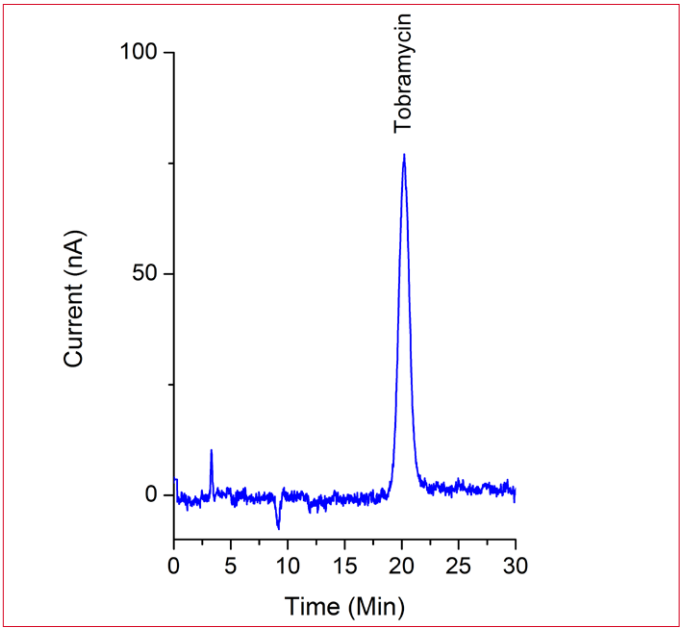

Figure 4: 20 µL injection of 2.5 µg/mL Tobramycin CRS in mobile phase (reference solution (b) as described in EP monograph).

Table 3

EP system suitability requirement		
Parameter	*EP criteria*	*Measured*
Resolution between Impurity A and Tobramycin	> 3.0	3.5
Signal-to-Noise ratio (Tobramycin)	> 10	17

The system suitability requirements are met for both parameters (table 3).

Linearity and repeatability

The linearity of Tobramycin and Kanamycin B (impurity A) was investigated in the concentration range of 10 - 50 µg/mL. For both components the correlation coefficients were better than 0.999 for peak areas. The relative standard deviation (RSD) in peak area for Tobramycin was determined for 8 replicate injections of test solution (b), which is a 0.1 mg/mL Tobramycin sample solution in mobile phase (see figure 4). The RSD was 0.7 % for the Tobra-mycin peak area.

Sample analysis

A commercial Tobramycin sample (CUD 621uA2B) was analyzed to determine the composition and related substances (impurities) using the acceptance criteria described in the EP monograph.

Assay

To determine the content (%) of Tobramycin in the sample the response of a 100 µg/mL Tobramycin sample solution (sample solution (b)) is compared to a 100 µg/mL Tobramycin CRS stand-ard (reference solution (e)) and the contents calculated. See figure 4 and table 4 below.

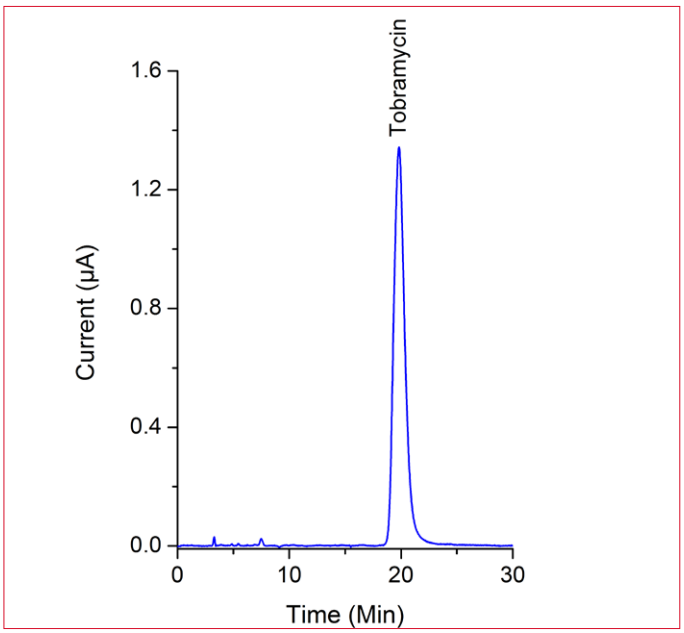

Figure 5: 20 µL injection of 100 µg/mL Tobramycin sample solution in mobile phase (sample solution (b) as described in EP monograph) for the Tobramycin assay analysis.

Table 4

Assay		
Sample	*EP criteria %*	*Measured**
Sample CUD 621uA2B	97-102	99.1

*) calculated on non-anhydrous sample

The contents was within the specified limits of the EP monograph.

Impurity analysis

To determine the impurity level in the sample, the responses of the impurity peaks of a test solution (a) containing a 1 mg/mL Tobramycin sample in mobile phase were compared to the response of the principle peak of reference solution (c). The chromatogram of test solution (a) is shown in figure 1.

Table 5

Impurity analysis Tobramycin sample CUD 621uA2B			
Impurity	*RRT**	*Peak Area (nA.s)*	*Discard#*
2	0.21	280	Y
3	0.24	58	Y
4	0.27	562	Y
5	0.30	103	Y
Neamine	0.38	663	Y
Nebramine	0.41	4327	N
8	0.53	137	Y
9	0.57	594	Y
Kanamycin sulphate B	0.80	843	Y

*) Relative retention time (RRT) with reference to Tobramycin (18.2 min).
#) Discard limit: any peak with an area less than that of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent) shown in figure 3.

The EP acceptance criteria for the amount of impurities are:

- *Any impurity:* Not more than twice the area of the Tobramycin peak in the chromatogram obtained with reference solution (c), and not more than 1 such peak having an area more than the area of the Tobramycin peak obtained with reference solution (c).
- *Total impurities:* Not more than 3x the peak area of the Tobramycin peak in the chromatogram obtained with reference solution (c).
- *Discard limit:* Impurities with peak areas smaller than the peak area of the principle peak (Tobramycin) in the chromatogram of reference solution (b) can be discarded.

Table 6

Results impurity analysis Tobramycin sample			
Impurity	*RRT*	*Relative Peak Area**	*EP criteria*
Nebramine (impurity B)	0.41	0.84	< 2
Total impurities*	-	0.84	< 3

*) The relative peak area of the impurity is calculated in the following way: Relative peak area = Peak area of the impurity divided by the peak area of the Tobramycin peak in the chromatogram obtained with reference solution (c).

In table 5 the peak responses (peak area in nA.s) are listed for all impurities found. Only the impurities with a response larger than the discard limit are taken into account in the calculation of the relative amount of impurities as specified under the limits section in the EP monograph. The results are shown in table 6.

The analyzed sample is in compliance with the acceptance criteria for both the contents and the impurity limits as set by the EP for Tobramycin and its impurities.

Conclusion

The ALEXYS Aminoglycosides Analyzer provides a suitable solution for the analysis of the composition & impurities in Tobramycin following the official method of the EP.

References

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3. J. Szunyog, E. Adams, E. Roets, J.Hoogmartens, 23, J. Pharm. Biomed. Anal., (2000) 891-896
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5. *Tobramycin in pharmaceutical preparations,* Antec application note, 217_014

Ordering information

180.0050W	Aminoglycoside Analyzer including Flowcell
250.1075	PLRP-S 1000 Å, 250x4.6mm, 8um

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