Advancing counterion analysis

Case Study

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Advancing Counterion Analysis:
A Practical HILIC–ELSD Approach for Modern Pharmaceutical Laboratories

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1. Abstract

Efficient and reliable counterion analysis remains a critical requirement in pharmaceutical development and quality control. This study demonstrates how Hydrophilic Interaction Liquid Chromatography (HILIC), coupled with Evaporative Light Scattering Detection (ELSD), can provide a flexible and cost-effective alternative to traditional techniques.

The approach enables the simultaneous separation and quantification of both anionic and cationic counterions, while also resolving the active pharmaceutical ingredient (API) free base from its associated salt. The findings highlight the potential of HILIC–ELSD as a practical tool for routine analytical workflows.

2. Introduction

Salt formation is a widely adopted strategy in pharmaceutical development, with many marketed drugs formulated as salts to enhance solubility and bioavailability. As a result, accurate quantification of counterions is essential not only for regulatory compliance but also for ensuring product quality and consistency.

Ion chromatography (IC) has traditionally been the method of choice for counterion analysis. However, increasing demand for efficiency, flexibility, and cost control is driving interest in alternative analytical approaches. This work explores HILIC–ELSD applicability as a robust and versatile platform for counterion determination.

3. Experimental

This development work is based on Waters application note “Method for the Analysis and Quantitation of Pharmaceutical Counterions Utilising Hydrophilic Interaction Liquid Chromatography”, with minor modification due to the dimensions of the column used.

The HILIC-based methodology has been demonstrated to provide effective separation of a wide range of counterions, offering good retention, resolution, and reproducibility. The method employs an ELS detector, which is well suited due to the lack of chromophores present within the counterion structures.

Table 1: Previously Reported Sample Results

API1 Ion chromatography	API2 AAS
Cl 7.0 %w/w	Na 0.15 %w/w

3.1 Instrumentation

Waters HPLC system with Waters 2424 ELS detector with Empower chromatography data system used.

HILIC column: Atlantis Premier BEH Z-HILIC VanGuard FIT Column, 5µm, 4.6mmx 100mm

3.2 Reagents/Materials

Mobile phases utilised in this experiment were as follows:

MP A-100% Acetonitile; MPB-Water, MPC-200mM ammonium format; MPD-2% Formic Acid; Diluent-60:40 v/v Acetonitrile:Water. Run time 16 min: Isocratic.

The salts used in this study were sodium chloride and sodium nitrate provided as certified 1 mg/mL standards from Supelco. The stock salt solutions were then appropriately diluted to prepare calibration standards for the dissolved counterions, covering the concentration ranges expected in the API samples, as outlined in Table 2.

Table 2: Sample and Ion Curve Concentrations

Nominal Sample Concentration (mg/mL)			
API1 (Cl)		API2 (Na)	
1.75		5.0	
Chloride Standard		Sodium Standard	
mg/mL	%	mg/mL	%
0.025	1.4	0.00250	0.05
0.050	2.9	0.00375	0.08
0.100	5.7	0.00500	0.10
0.125	7.1	0.01000	0.20
0.050	8.6	0.01500	0.30

4. Results and Discussion

4.1 Chromatography

The analysis of the two counterions in this study required isocratic chromatographic conditions to achieve complete separation. Typical standard and sample chromatograms are presented in Figure 1 to Figure 4.

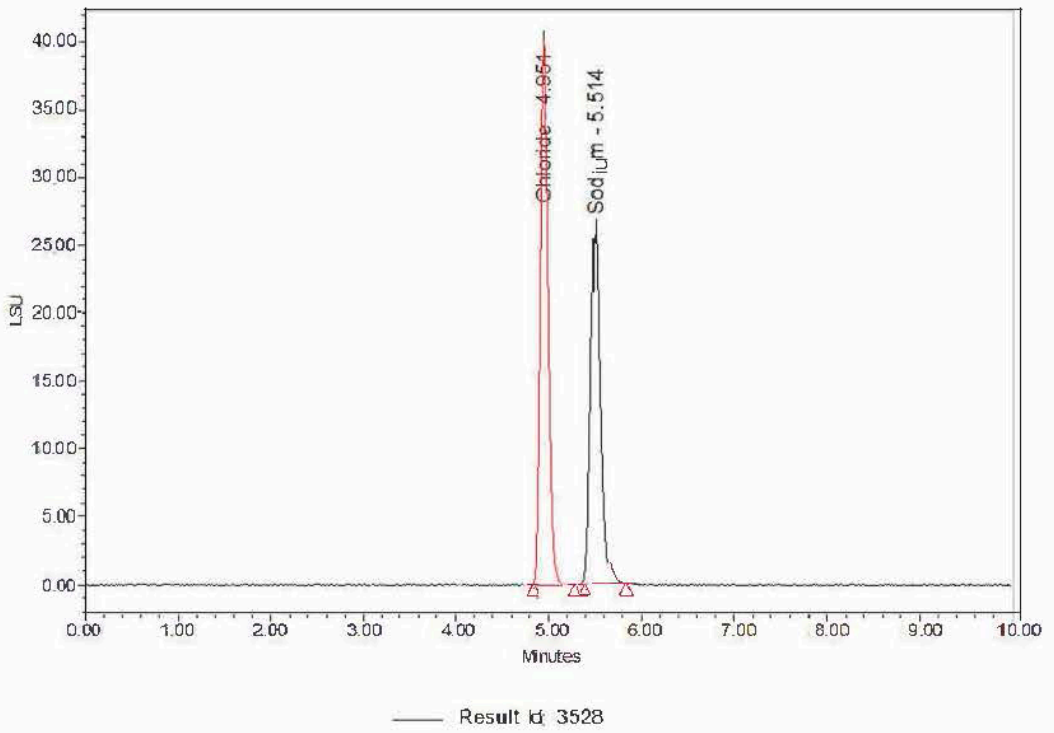


Figure 1: Chloride Standard Example (NaCl used)

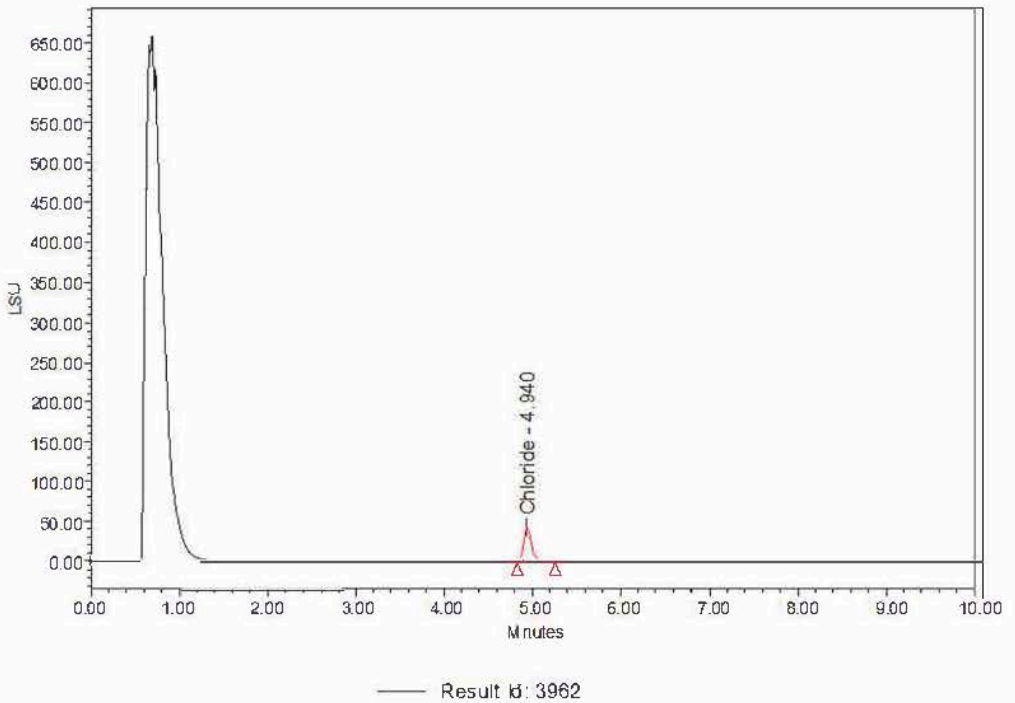


Figure 2: Chloride Ion in the Sample (API1)

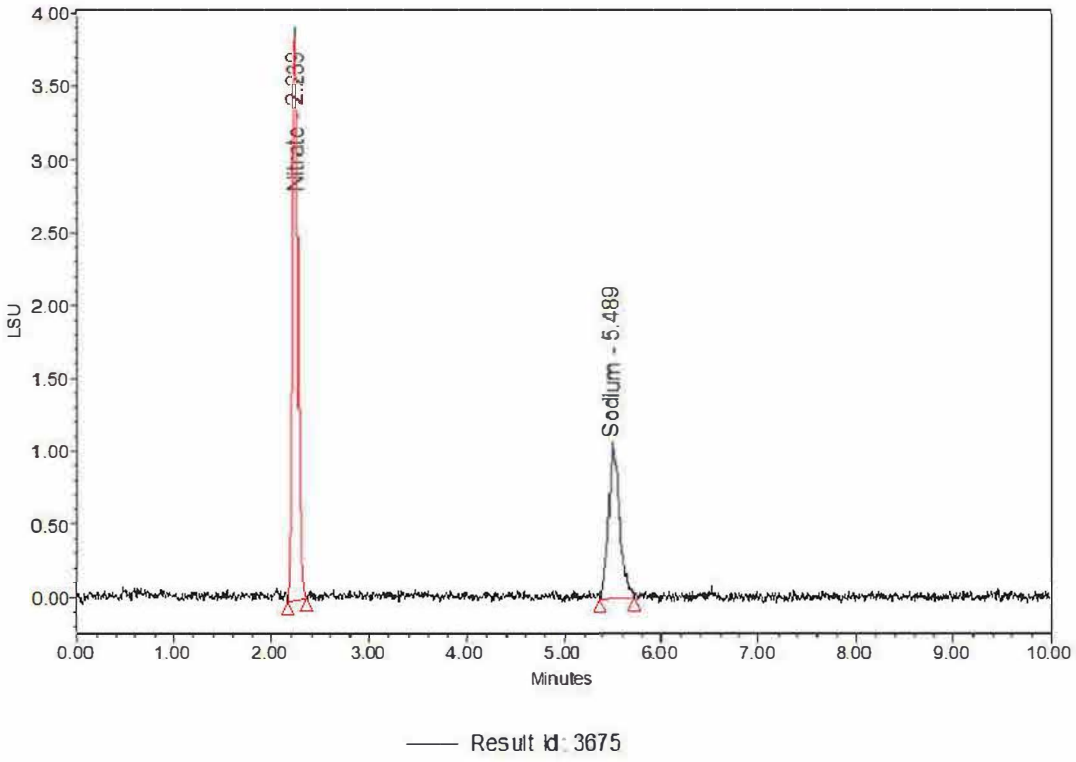


Figure 3: Sodium Standard Example (NaN03 used)

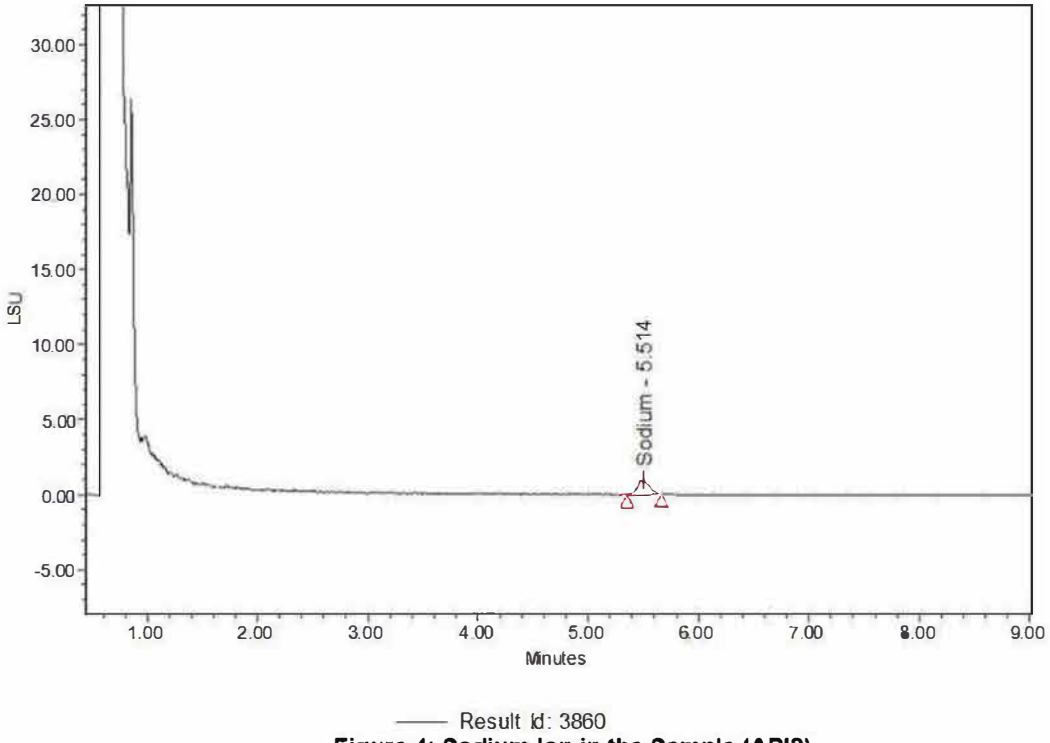


Figure 4: Sodium Ion in the Sample (API2)

To ensure the method was suitable for routine analysis, method suitability was demonstrated by performing an assessment of linearity and recovery at the API specification level for each ion.

4.2 Linearity assessment

Linearity for each counterion was evaluated using one or more of the loglog, quadratic, and cubic calibration models to account for the non-linear response characteristics of ELSD. Selecting the most appropriate calibration model is critical to achieving an accurate fit across the concentration range evaluated.

4.2.1 Chloride Data

Calibration curves were generated in Empower 3 (Figures 5 and 6). For chloride, both log-log and cubic calibration models were tested. The selected calibration curves were subsequently used to quantify the counterion in the API sample. The results for sample content and recovery are presented in Table 3.

The R2 values for the chloride counterion calibration curves were both ≥0.998 demonstrating excellent model fit. Recovery data (Table 3) indicate that both calibration models provide accurate and precise quantitation of the counterion. The calculated results were in close agreement with the ion chromatography (IC) result presented in Table 1 (7.0% w/w), demonstrating the suitability of the method for the quantitative analysis of chloride counterions in pharmaceutical compounds.

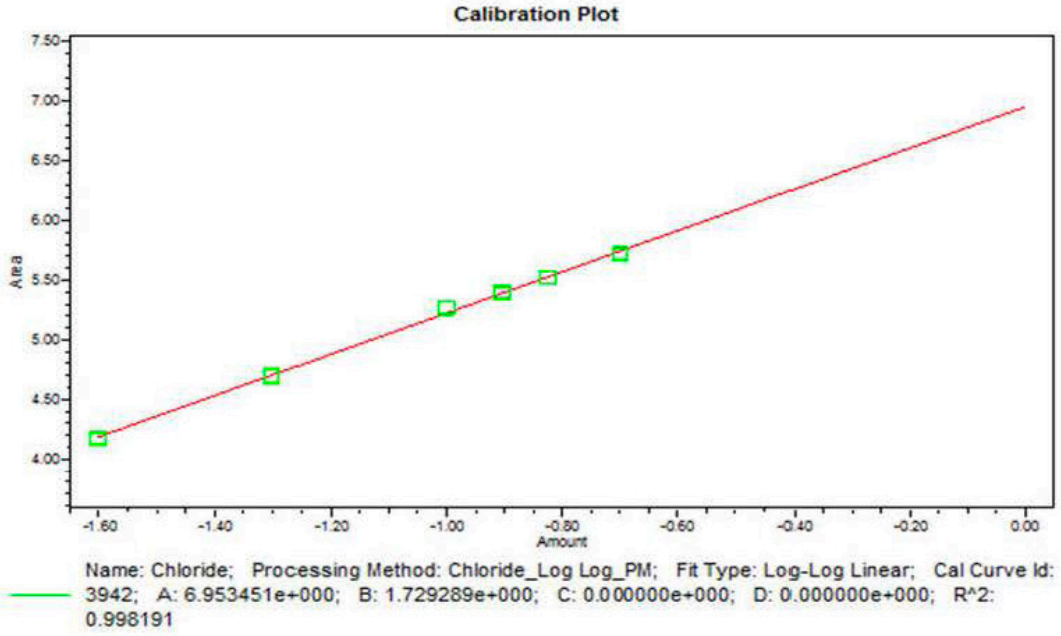


Figure 5: Chloride Ion Log-Log Linearity plot

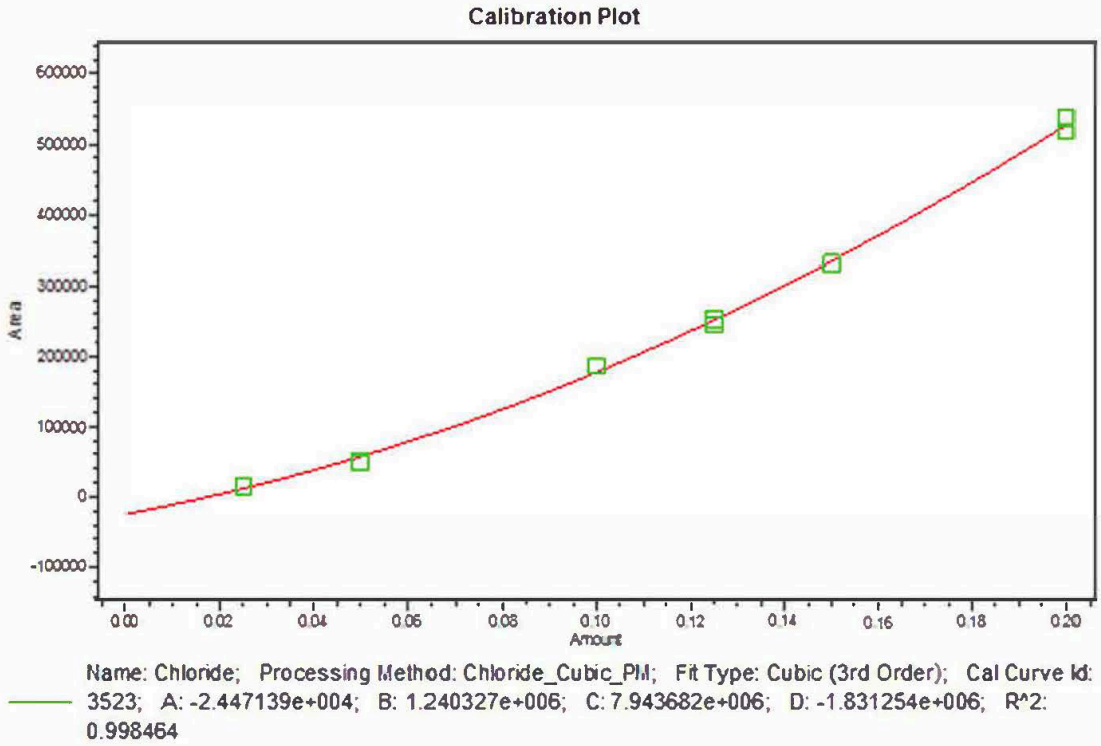


Figure 6: Chloride Ion Cubic Linearity Plot

Table 3: Chloride Sample Results

	Chloride	
	Log-Log Fit	Cubic Fit
R^2	0.998	0.999
Sample %w/w P1	7.1	7.0
Sample %w/w P2	7.3	7.2
Sample %w/w P3	7.0	6.9
Sample %w/w Mean	7.1	7.0
%RSD	2.1	2.2
Sample % Recovery P1	99.4	100.1
Sample % Recovery P2	100.7	101.5
Sample % Recovery P3	97.8	98.2
Sample % Recovery Mean	99.3	99.9
%RSD	1.5	1.6

4.2.2 Sodium Data

Calibration curves were generated in Empower 3 (Figures 7 to 9). For sodium, log-log, quadratic and cubic linear models were tested. The selected calibration curves were subsequently used to quantify the counterion in the API sample. The results for sample content and recovery are presented in Table 4.

Although the cubic regression model produced a slightly higher R^2 value for the sodium calibration curve than the other models evaluated, inspection of Figure 7 demonstrates that, over this narrow concentration range, a cubic fit is not justified and results in overfitting without providing a meaningful improvement in accuracy.

A quadratic regression model was considered the most appropriate fit for the data, although the log-log model produced comparable results. This comparison highlights the importance of critically assessing calibration models rather than relying solely on statistical indicators such as R^2 values. The results obtained were both accurate and precise and showed good agreement with the sodium content determined by AAS (0.15% w/w). These results demonstrate that the method is suitable for the accurate and reliable quantitation of sodium counterions in pharmaceutical compounds.

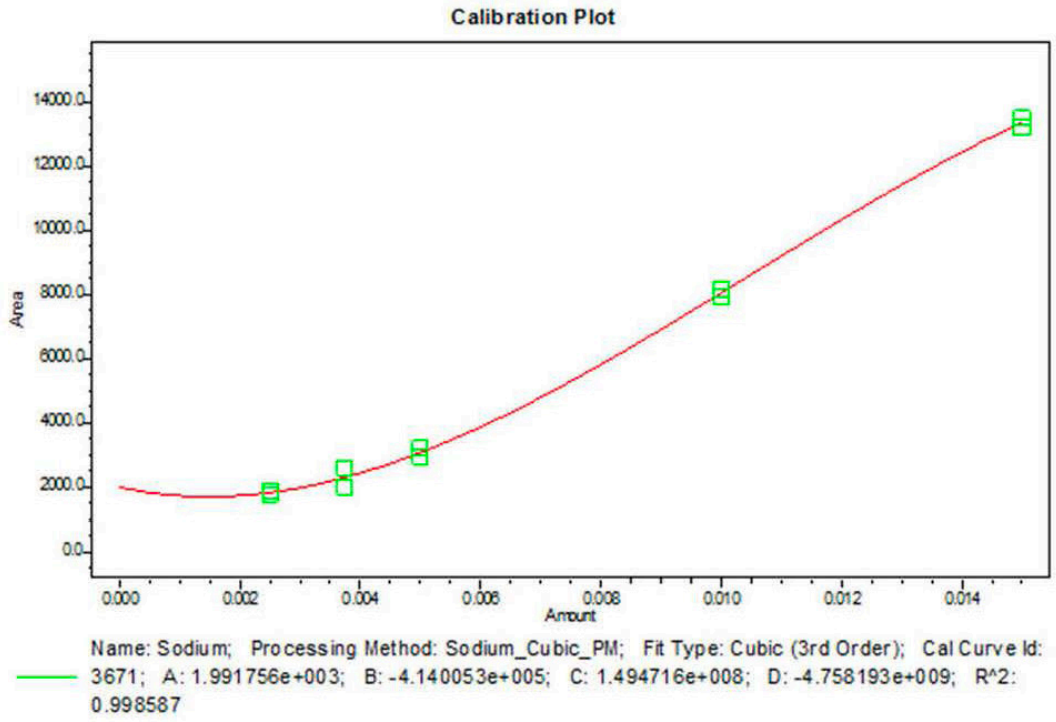


Figure 7: Sodium Cubic Linearity plot

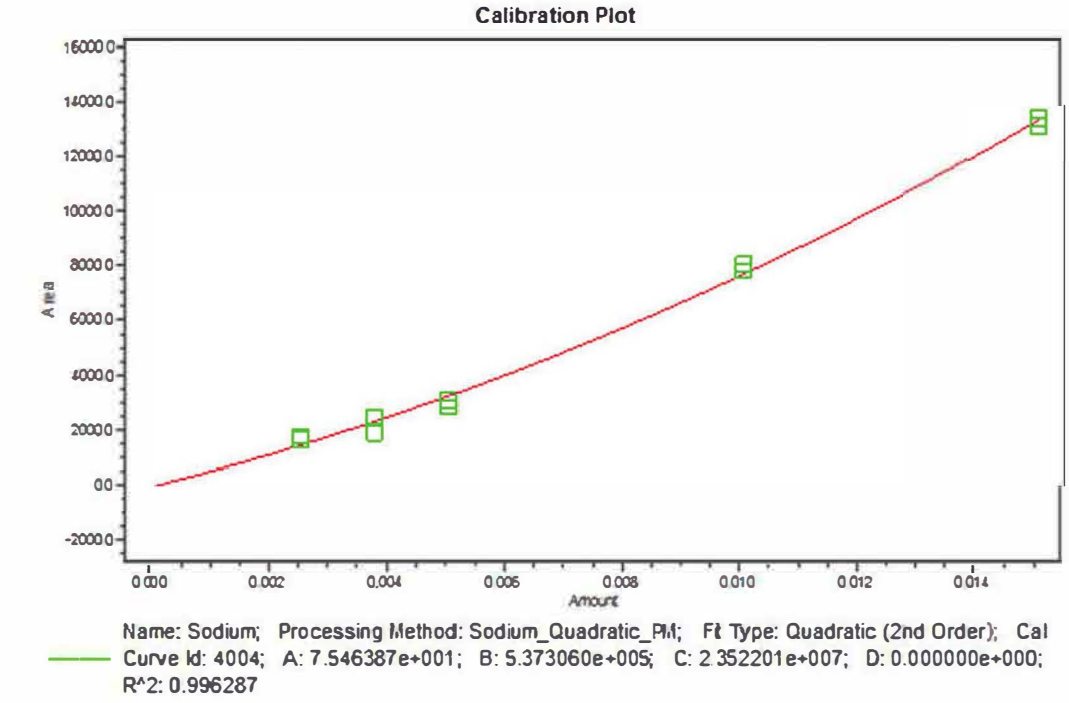


Figure 8: Sodium Quadratic Linearity plot

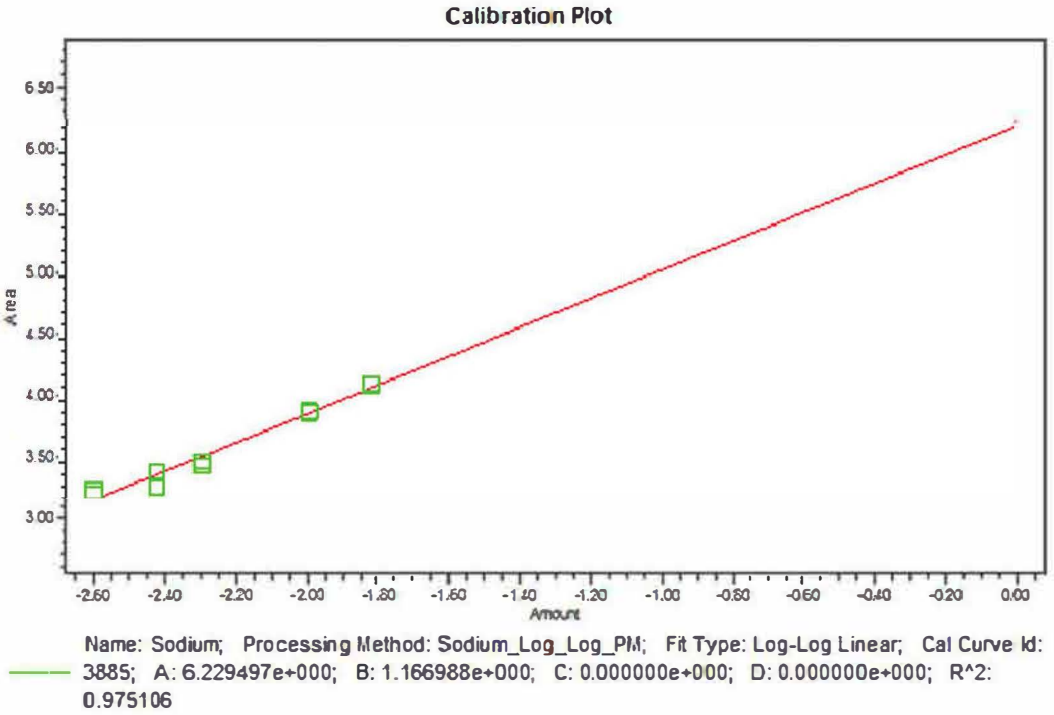


Figure 9: Sodium Ion Log-log plot

Table 4: Sodium Sample Results

	Sodium		
	Log-Log Fit	Quadratic Fit	Cubic Fit
R^2	0.975	0.996	0.999
Sample %w/w P1	0.18	0.18	0.17
Sample %w/w P2	0.16	0.17	0.17
Sample %w/w P3	0.15	0.16	0.16
Sample %w/w Mean	0.16	0.17	0.17
%RSD	9.4	5.9	3.5
Sample % Recovery P1	93.6	102.7	104.8
Sample % Recovery P2	98.0	101.8	109.4
Sample % Recovery P3	98.8	97.2	110.2
Sample % Recovery Mean	96.8	100.6	108.1
%RSD	2.9	2.9	2.7

5. Conclusion

The HILIC-ELSD results showed excellent agreement with ion chromatography and AAS data, demonstrating accurate and reproducible quantitation of chloride and sodium as pharmaceutical counterions.

In addition to providing effective separation of the counterions from their associated APIs, the methodology offers a straightforward analytical workflow with flexible mobile phase options and minimal susceptibility to non-volatile buffer contamination. While appropriate calibration model selection is essential because of the non-linear response characteristics of ELSD, the study demonstrates that accurate quantitation can be readily achieved. Taken together, these findings show that HILIC coupled with ELSD represents a robust and practical alternative for routine pharmaceutical counterion analysis.

While this study focused on the quantitation of chloride and sodium counterions, previous literature has demonstrated the applicability of HILICELSD to a broader range of inorganic ions. Further evaluation of additional pharmaceutical counterions could establish the methodology as a versatile platform method, capable of supporting multiple development programs using a common analytical approach. Such a platform strategy could reduce method development effort, improve consistency across projects, and provide an efficient alternative to maintaining multiple counterion-specific analytical techniques.

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