Targeted and non-targeted analysis of a potential contaminant in dog food

Introduction

Food safety remains one of the most critical challenges facing manufacturers today. With the global food industry valued at US$4.93 trillion industry in 2025 and expected to grow annually by a five-year compound annual growth rate (CAGR) of 6.41%,[1] ensuring product quality has never been more important. Consumer trust hangs in the balance—food scares and recalls can devastate even well-established brands overnight.

The pet food sector presents its own unique challenges within this landscape. At US$158 billion globally with a five-year CAGR of 5.79%,[2] the pet food market has evolved far beyond simple nutrition. Pet owners demand premium products that meet the same safety standards they expect in human food. Dog food is a significant portion of this with growth in premium and specialty product segments, with owners increasingly concerned that they are providing their dogs with food that is both nutritious and free of dangerous contaminants.

Traditional analytical methods often fall short when faced with the complexity of modern food matrices. Liquid chromatography-mass spectrometry (LC-MS), however, is ideally suited to analyzing food samples due to its ability to efficiently separate compounds and the specificity of the detection technique. Whether analyzing human food or pet food, it can detect a large number of compounds across a broad range of molecular weights, ensuring stringent safety standards are met.

Given these demanding analytical requirements, Wiley's KnowItAll[3] provides an integrated solution to identify, analyze, and manage analytical data from food samples. It supports multiple techniques and vendor formats, with its LC Expert software providing automated non-targeted analysis (NTA) capabilities. Users can also draw or import a structure for targeted analysis in the integrated ChemWindow application.

This application note demonstrates how both approaches—targeted and non-targeted analysis—can work together to provide comprehensive food safety assessment. We investigated a dog food sample measured using LC-MS/MS. It was first analyzed using a targeted workflow from a compound structure that preliminary investigation had highlighted as a potential contaminant. Next, it was searched against a collection of tandem MS libraries which also detected the contaminant. These results showcase not only the technical capabilities, but also how an integrated targeted and non-targeted workflow can provide confidence levels required for food safety decision making.

Experimental

Our investigation centered on a dog food sample likely to raise concerns during routine quality control screening.

- **Sample Analysis:** The samples were Thermo Q Exactive Orbitrap data files acquired in data-dependent acquisition (DDA) mode.
- **Software:** Data analysis was performed using Wiley's KnowItAll Analytical Edition (Version 2025) software with the LC Expert, ChemWindow, and ReportIt applications.
- **Database:** The Maurer/Meyer/Helfer/Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites[4], the NIST Tandem Mass Spectral (MS/MS) high resolution libraries[5], and the Wiley Registry of Tandem Mass Spectral Data – MSforID[6]* were used for non-targeted compound identification.

Results and Discussion

Opening the raw LC-MS/MS data file in **LC Expert*** revealed the complexity of food matrices. The software automatically deconvoluted the data, presenting it as clearly defined Extracted Ion Chromatogram (EIC) peaks in the LC panel. Each component appeared in the **Peaks** table along with characteristic information: retention time (RT), peak area, and peak height–essential parameters. We zeroed in on the region between 0.2 and 3.0 minutes retention time using the **Include Range Bar** for a more detailed investigation (Figure 1).

Targeted analysis: Following a lead

Prior examination of the data had raised concerns about a specific compound: allopurinol. This discovery was particularly concerning because allopurinol, while prescribed to dogs for kidney stones, should not typically be found in dog food[7]. Its presence could indicate either contamination or adulteration—both serious food safety issues.

To perform a targeted accurate mass search, a structure of allopurinol was first drawn in **ChemWindow**, KnowItAll's integrated structure drawing application (Figure 2). It also includes tools to calculate the structure's accurate mass and isotopic distribution, critical parameters for targeted mass spectrometry searches starting from a chemical structure.

With our molecular target defined, we initiated an accurate mass search of the dog food data file by seamlessly transferring the structure information to **LC Expert** and targeted accurate mass search results for the previously selected range opened in LC Expert (Figure 3). The integration between applications exemplifies how modern analytical software can eliminate the tedious manual data transfer in
multi-step analyses.

The targeted search results clearly demonstrated that allopurinol was indeed present in the dog food sample. The software identified peaks at the expected retention times providing rapid, definitive evidence of the potential contaminant.

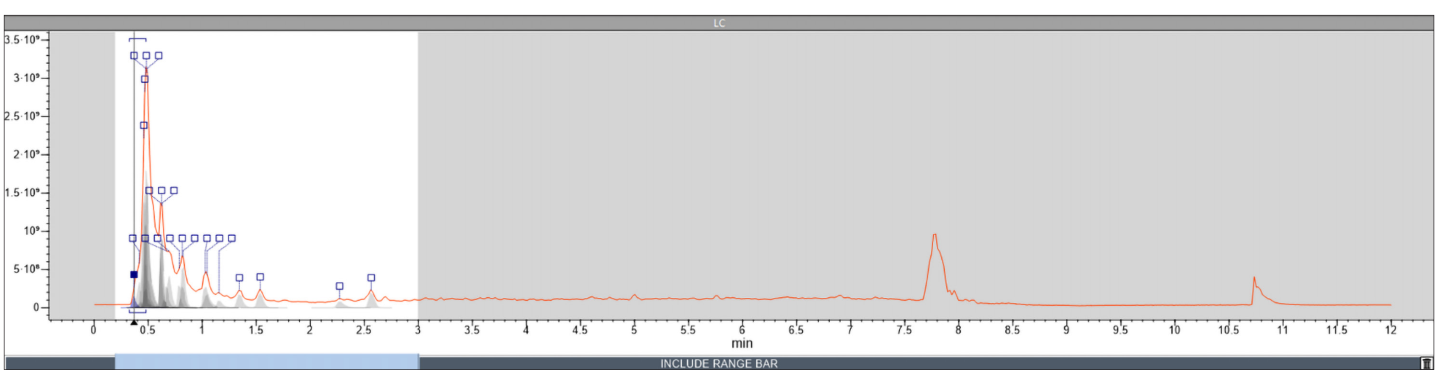


***Figure 1.** This is the region that was selected for analysis in the chromatogram using the **Include Range Bar**.*

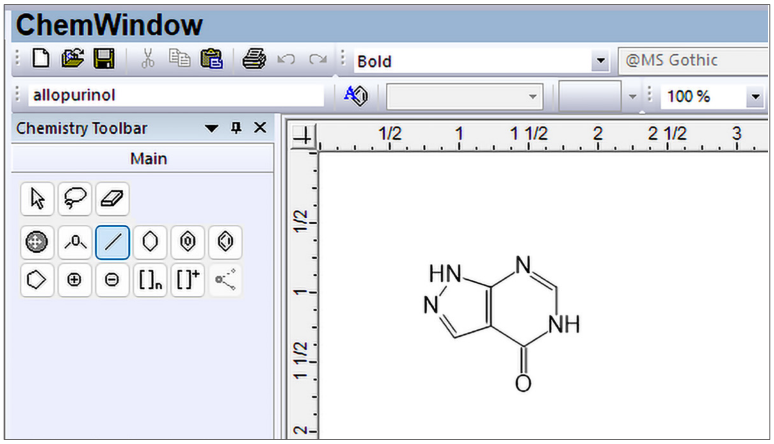


Figure 2. *The chemical structure of allopurinol drawn in the* ***ChemWindow*** *application.*

Peaks											
	RT [min]	Peak Area	Peak Area [%]	Peak Height	FWHM [	Base Ion [*m/z*	Annotated Name	Molecular Formula	Match Score	Mass Accuracy [ppm	Calculate
5	0.4766	50052460	11.60	1079363746.9	0.0326						
6	0.4829	84119437	19.49	1787580000.9	0.0320						
7	0.4909	58030601	13.44	1629017459.3	0.0342						
8	0.6164	28378121	6.57	647322651.3	0.0283						
9	0.6240	27944821	6.47	898968267.7	0.0327	137.0457	Allopurinol	$C_5H_4N_4O$	99.69	-0.64	137.0458
10	0.6285	15699503	3.64	881744722.8	0.0317	137.0457	Allopurinol	$C_5H_4N_4O$	99.68	-0.64	137.0458
11	0.6691	9935876	2.30	272907980.9	0.0423						

Figure 3. *Allopurinol was detected in the dog food sample using an accurate mass search initiated from ChemWindow.*

Non-targeted confirmation: Casting a wider net

Whilst the targeted analysis confirmed our suspicions about allopurinol, we recognized the value of further validation using non-targeted screening. Using this approach we can search the entire dataset against commercially available high-resolution accurate mass (HRAM) databases—a powerful complement to our targeted workflow. The **Database Search** panel displayed the search progress and MS/MS spectral matches with their scores and MS1 centroid RT (Figure 4). The results were striking: Allopurinol appeared as the top four hits, all with high confidence scores. Most significantly, these non-targeted searches coincided with targeted accurate mass search (0.6240 and 0.6285 minutes), providing robust cross-validation.

Database Search

RT [min]	#	Match	Score	HQI	R.HQI	Notes
0.3691	1	DL-Arginine	98.18	93.54	98.69	
0.4163		No match found				
0.4595		No match found				
0.4682	1	4-Aminopentanoic acid	98.48	93.93	98.99	
0.4766	1	4-Aminopentanoic acid	98.48	93.93	98.99	
0.4829		No match found				
0.4909		No match found				
0.6164	1	Allopurinol	98.56	93.90	99.08	
0.6240	1	Allopurinol	98.56	93.90	99.08	
	2	Allopurinol	97.52	92.76	98.04	
	3	Allopurinol	96.65	91.93	97.17	
	4	Allopurinol	95.46	93.31	95.70	
	5	Hypoxanthine	95.38	93.24	95.62	
	6	2'-Deoxyinosine	94.93	92.94	95.15	
	7	6-Butoxy-9H-purine	94.71	92.73	94.93	
	8	Hypoxanthine	94.04	92.07	94.26	
	9	2',3'-Isopropylideneinosine	93.61	92.36	93.75	
	10	Allopurinol	90.52	88.72	90.72	
0.6285	1	Allopurinol	98.56	93.90	99.08	
0.6691	1	4-Pyridinecarboxylic acid	98.51	98.12	98.56	
0.6884	1	Adenine	98.96	95.25	99.37	
0.7875		No match found				
0.8154	1	Nicotinamide	98.87	97.47	99.03	
0.8226	1	Nicotinamide	98.87	97.47	99.03	
1.0285	1	tert-Butyl (1S,4S)-2,5-diazabicyclo[2.2.1]he...	37.64	0.12	41.81	
1.0460	1	tert-Butyl (1S,4S)-2,5-diazabicyclo[2.2.1]he...	37.64	0.12	41.81	
1.1560	1	Thiamine	94.62	62.35	98.21	
1.3441	1	DL-Indole-3-lactic acid	96.06	94.47	96.23	
1.5363		No match found				
2.2710	1	N6-Methyladenosine	97.60	97.17	97.65	
2.5638	1	Nefiracetam	83.69	16.34	91.18	

Figure 4. *The* ***Database Search*** *panel displays matches in the MS2 spectrum, along with their scores.*

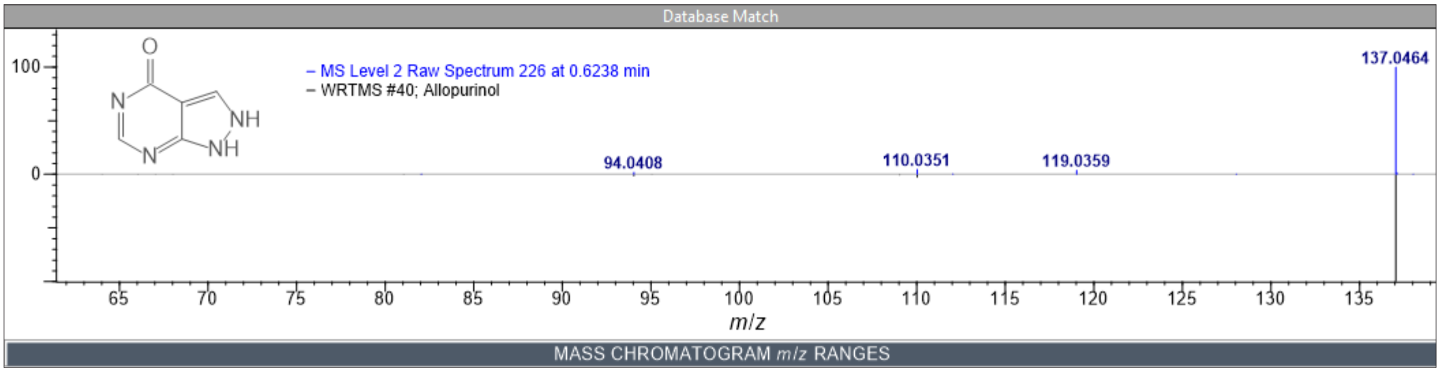
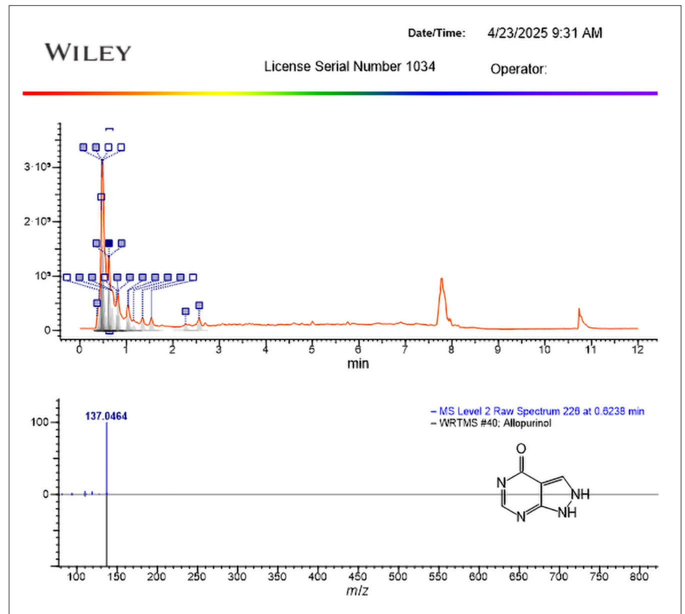


Figure 5. *The **Database Match** panel displays the matched spectra and a structure for the hit.*

The **Database Match** panel demonstrated the quality of the spectral matches, displaying the raw spectrum against the best match from the database, including the MS2 scan's RT, Database Abbreviation, and its Record ID as well as a structure, if available (Figure 5). The close correspondence between the sample and reference spectra, combined with the structural information retrieved from the databases, left no doubt about the compound's identity.

Documentation and reporting

The analysis workflow concluded with the generation of a comprehensive report documenting the findings using the automatic report generation tools available across all KnowItAll applications, including LC Expert. LC Expert's integration with the ReportIt application enabled direct transfer of results to a standardized template with a single click. The final output, formatted into a standard template, and saved as a Portable Document Format (PDF) for further review (Figure 6), demonstrates how KnowItAll's integrated reporting functionality streamlines documentation requirements while maintaining the consistency and completeness necessary for compliance and quality assurance protocols.



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	3	Allopurinol	96.65	91.93	97.17	
	4	Allopurinol	95.46	93.31	95.70	
	5	Hypoxanthine	95.38	93.24	95.62	

Figure 6. *Automated report generation using a standard showing compound identification results.*

Conclusion

This investigation illustrates the power of integrated analytical workflows in addressing real-world food safety challenges. The seamless combination of targeted and non-targeted approaches within KnowItAll's unified platform provided multiple lines of evidence confirming allopurinol contamination in the dog food sample.

The targeted analysis, initiated from a drawn chemical structure, provided rapid confirmation of suspected contamination. The independent non-targeted database screening not only validated these findings but also demonstrated the comprehensiveness of the analytical approach. The correlation of results from both methods provides the level of certainty required for food safety decisions. In this case, there was higher confidence that the allopurinol was present in the sample, due to our dual workflow.

Perhaps equally important is how this integrated workflow streamlined the entire analytical process. From initial data exploration through final report generation, analysts remained within a single software environment, eliminating the inefficiencies and potential errors associated with multiple software packages and manual data transfers.

For food safety laboratories facing increasing analytical demands, this case study shows how integrated platforms like Wiley's KnowItAll can enhance both the speed and reliability of contamination detection. Whether investigating known suspects through targeted analysis or screening for unexpected contaminants through non-targeted approaches, the combination provides comprehensive coverage that helps ensure the safety of both human and pet food products.

The detection of allopurinol in this dog food sample serves as a reminder that stringent food safety measures must extend across all sectors of the food industry. As analytical capabilities continue to advance, so does our ability to protect the safety of both human and pet food products.

References:

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∗Subscription required to Maurer/Meyer/Helfer/Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites database, the NIST MS/MS Mass Spectral Libraries, Wiley Registry of Tandem Mass Spectral Data – MSforID, and the LC Expert application.