

Rapid Confirmation of the Purity of APIs Using the Agilent LC/MSD iQ Mass Selective Detector and WalkUp Software

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Abstract

This application note demonstrates how the addition of mass information to a UV-based method can greatly improve the efficiency of chromatography. With added mass information, coeluting species can be easily distinguished in fast chromatographic methods, especially if those compounds respond poorly to UV detectors or do not contain a chromophore. In some cases, detection of trace compounds can be achieved at levels 100 times better than UV only-based workflows. For this application note, 22 different active pharmaceutical ingredients (APIs) were analyzed for their purity in an open-access environment using Agilent MassHunter WalkUp with the Agilent InfinityLab LC/MSD iQ system. The InfinityLab LC/MSD iQ is a self-aware, robust mass detector that adds mass information to standard HPLC workflows. MassHunter WalkUp is software that enables an LC/MS system to be used in an open-access environment.

Introduction

Synthesis of an API requires many monitored reaction steps. To ensure that a reliable and cost-efficient synthetic pathway is established, each reaction step must be tested for overall purity and yield. Obtaining fast results is key to determining appropriate decisions for the next step. With the addition of mass information, faster liquid chromatography separation methods can be implemented because mass detection enables the identification of analytes that are coeluting. Furthermore, the need for running standards, which is required if UV detection is used, can be reduced or eliminated. Additionally, some labs test the purity of the API by collaborating with an outside analytical group due to the complexities of implementing methods involving mass spectrometry detection. The InfinityLab LC/MSD iQ single quadrupole mass spectrometer along with the MassHunter WalkUp software eliminates these complexities by providing an intuitive open-access system with mass confirmation.

The InfinityLab LC/MSD iQ single quadrupole mass selective detector (MSD) demonstrates exceptional reliability, features smart diagnostics, and can handle low to high flow rates. It can also automatically optimize MS parameters to provide optimal results under an intelligent and automated acquisition mode called Auto Acquire.

Chromatographers can adopt the system by simply adding it to their existing Agilent 1260 Infinity II or 1290 Infinity II LC stack. It can also be integrated seamlessly into the older generations of Agilent LC stacks.

With MassHunter WalkUp software, the instrument can be used as an open-access system. There are two types of WalkUp users:

- A system administrator
- Sample submitters

Methods are controlled by an administrator, enabling sample submission in a controlled environment. Since WalkUp operates above the acquisition and data analysis software, the submitter does not need prior knowledge of Agilent OpenLab CDS to operate the instrument. They only need to submit a sample using WalkUp's Rapid Sample Submission user interface that requires virtually no training to use, as illustrated in Figure 1.

The screenshot displays the 'Rapid Sample Submission' interface. At the top, the title 'Rapid Sample Submission' is on the left, and 'Queue Runtime' with a value of '0' is on the right. The interface is divided into two main sections. The left section contains a sidebar with 'Active Samples' and 'Clear Tray' buttons. The main area has input fields for 'User Name' (filled with 'chemist'), 'Password' (masked with dots), 'Sample Name' (filled with 'Amitriptyline'), 'Sample Count' (a numeric field with '1' and '+'/'-' buttons), 'WalkUp Method' (filled with 'Sample Purity'), and 'Mass Confirmation' (filled with '278'). Below these fields are 'Submit' and 'Cancel' buttons. The right section is titled 'Select Method' and shows 'Sample Purity' as the selected method, with an information icon and a note '3 min ACN gradient'. At the bottom of the interface, a message states: 'Please contact WalkUp Administrator in case of any errors/warnings. No Samples in Queue'.

Figure 1. The Rapid Sample Submission user interface of Agilent MassHunter WalkUp software. Sample submitters only need to log in and select an analytical method with the target mass/name. The measurement and data analysis automatically occur in the background; submitters will receive a PDF report of their results through email.

Experimental

Standards and chemicals

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile was purchased from Honeywell (Morristown, NJ, USA). All API compounds were purchased from Millipore-Sigma (Merck, Darmstadt, Germany). Ultrapure water was produced from a Milli-Q Integral system equipped with an LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA).

Instrumentation

The analysis system consists of the following modules:

- Agilent 1290 Infinity II High Speed Pump (G7120A)
- Agilent 1290 Infinity II Vialsampler (G7129B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)
- Agilent InfinityLab LC/MSD iQ (G6160A)

The DAD with an Agilent 60 mm Max-Light cartridge flow cell (G4212-60007) was used to maximize the sensitivity of the UV detector.

Sample preparation

API standards were weighed into 15 mL falcon tubes, and diluted to a concentration of 10 µg/mL with either ACN or MeOH depending on their solubility. The samples were analyzed immediately, and left in solution at room temperature for over three months to simulate storage degradation. The APIs were analyzed with their nominal mass selected as the target mass for a sample purity analysis.

Table 1. List of API compounds.

API	Nominal Mass (Da)		
Amitriptyline	277	Buspirone	385
Erythromycin	733	Clozapine	326
Captopril	217	Cetirizine	388
Trazodone	371	Paclitaxel	853
Paroxetine	329	Aspirin	180
Clopidogrel	321	Loratadine	382
Fosinopril	563	Fexofenadine	501
Nefazodone	469	Phenytoin	252
Zofenopril	429	Caffeine	194
Norethindrone	298	Ibuprofen	206
Carboplatin	371	Omeprazole	345

Table 2. Agilent 1290 Infinity II LC method parameters.

Parameter	HPLC Set Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm at 40 °C (p/n 699675-902)
Mobile Phase A	0.1% FA in H ₂ O
Mobile Phase B	0.1% FA in ACN
Gradient	Time (min) %B
	0.00 5
	1.75 90
	2.90 90
3.00 5	
Post Run	0.7 minutes
Flow Rate	0.8 mL/min
Injection Volume	1 µL
Detection UV	[254,5/ref. 360,80] nm

Table 3. Agilent InfinityLab LC/MSD iQ parameters established automatically by Auto Acquire.

Parameter	LC/MSD iQ Set Value
Ion Source	ESI±
Acquisition Mode	Auto Acquire mode
Points/Second	2
Scan Range	m/z 100 to 1,000

OpenLab CDS

Agilent OpenLab CDS software was used for data acquisition, processing, and reporting. OpenLab CDS provides compliance features that support data integrity in accordance with US FDA 21 CFR Part 11, EU Annex 11, and other similar regulations. The 1290 Infinity II LC and LC/MSD iQ are designed to ensure reliable and robust LC/MS for routine applications in GxP laboratories.

MassHunter WalkUp software

MassHunter WalkUp is open-access Agilent software that is an add-on to OpenLab CDS, ChemStation, and MassHunter acquisition platforms. WalkUp is control software that sits above the regular data acquisition, and provides a simple user interface to submit samples.

Results and discussion

Extra MS information for sample purity assessments

Adding an MSD to UV detection in an HPLC workflow enables users to add another dimension of information to their analysis. In addition, the MSD can detect compounds that absorb poorly in the UV or that do not contain an active chromophore. It also provides unambiguous information on coeluting analytes, which UV detection alone cannot discern. Identifying impurities is easier with mass information, negating the need for analytical standards or the time associated with running those standards to determine retention times. This allows fast sample purity assessments without the knowledge of expected retention times.

It is known that the API erythromycin does not absorb well in the UV spectrum. Impurities are detected at a different wavelength than the API. In many cases, impurity standards or organic reaction intermediates are hard to obtain. In addition, the absorption of the API at 210 nm is almost indistinguishable from the noise. This can only be confirmed by a standard being measured to find the expected retention time.

Figure 2 shows that the utility of mass detection can be seen in the MS chromatogram, which has a large peak identified as erythromycin, based on its nominal mass (733 Da). Without the mass information, there is ambiguity as to which UV peak is an impurity and which is the API.

Sample Purity Results

Target mass	Target found in analysis	Purity (%)	Purity result
733.0	Yes	85.44	Impure

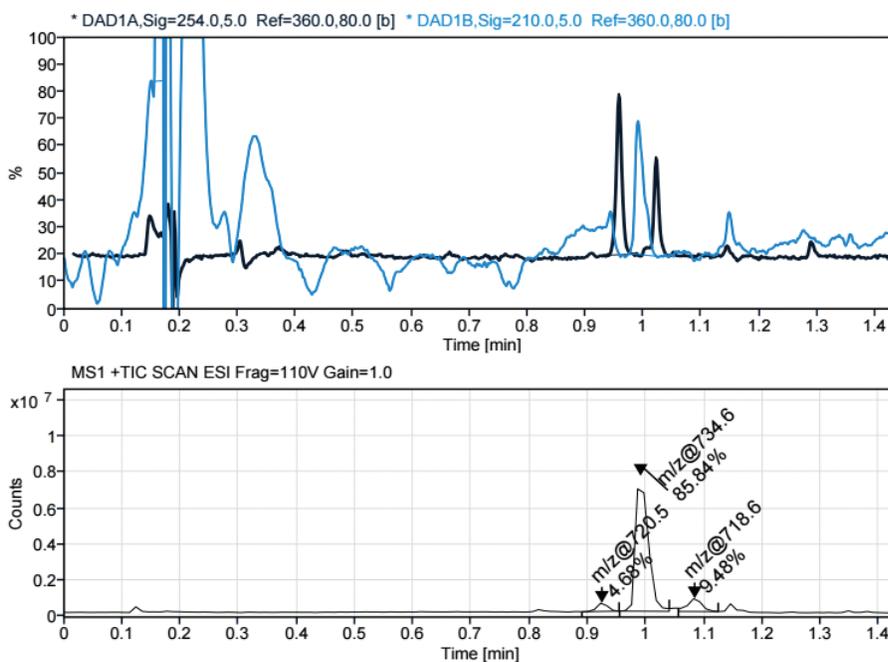


Figure 2. Sample purity results for an erythromycin sample. The upper graphic shows the UV chromatograms at 254 and 210 nm (black and blue traces respectively), and the lower graphic is the MS chromatogram with the base peak m/z and peak area % labeled. From the MS data, it can be confirmed that the chromatographic peak at 210 nm is the API (~1.0 minutes) and the two peaks detected at 254 nm are impurities.

Streamline reaction monitoring with OpenLab CDS sample purity workflow

OpenLab CDS provides built-in workflows that automatically process data and generate reports. With established methods, samples submitters do not need to perform data analysis, saving valuable time. The sample purity workflow allows submitters to enter up to five target masses to monitor during a measurement. After the measurement is complete, data processing occurs automatically with the specified adducts of the target mass extracted from the scan signal. Automatic data processing then finds the matching peak in the scan signal based on the summed EIC retention time of the target adducts. If a target is found, it will be displayed as "found" in the report, and the following calculations are performed to determine sample purity based on either the TIC % or EIC/TIC %.

For this application note, the positive TIC % MS sample purity workflow was used with $[M+H]^+$ and $[M+Na]^+$ target adducts selected, as shown in Figure 3. In addition, the $[M-H]^-$ and $[M+HCOO]^-$ adducts were selected for the negative scan mode.

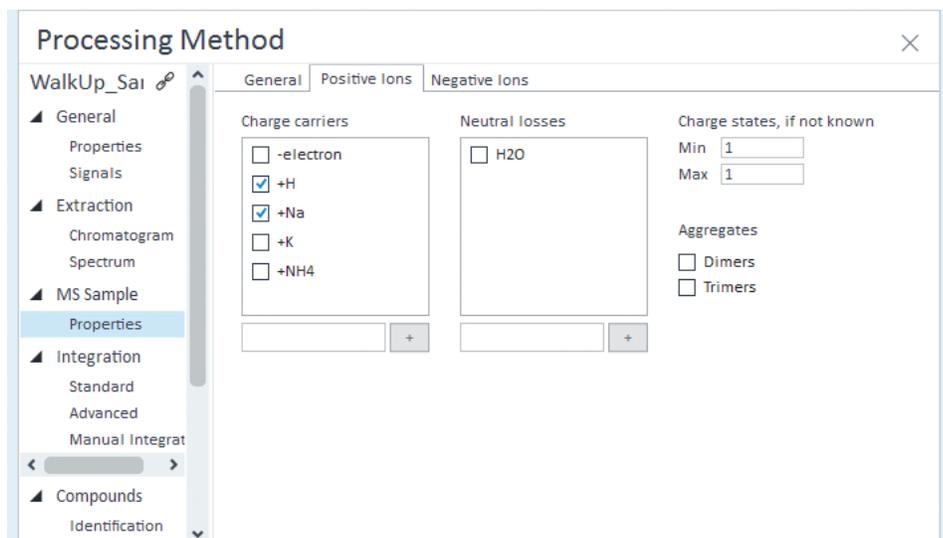


Figure 3. MS sample purity processing method parameters within the Agilent OpenLab CDS Data Analysis processing method tab. The MS sample purity was determined by TIC % calculation. H^+ and Na^+ adducts were selected for positive scan mode. $HCOO^-$ adduct and the deprotonated molecule were selected for the negative scan mode. Once the parameters are saved, data processing and report generation occurs automatically for all measurements.

Figure 4 shows OpenLab data analysis and the sample purity results of all 22 APIs. All the injections can easily be viewed together, and APIs that did not meet the minimum purity limit or were not detected are flagged in red. Sample submitters receive a sample purity report for each API, similar to those in Figure 6.

Mass information is integral for deducing chemical reactions

Being able to monitor reactions throughout the synthetic pathway is key for making proper decisions and adjustments if necessary. Deciding on the best course of action requires fast and reliable results. This simulated degradation study started with a dilution of fresh captopril, which shows one peak in the UV chromatogram when measured at 210 nm. This is also confirmed as the API based on the mass information. The MS chromatogram only contains one peak, with the EIC of the target mass matching the single peak; thus the sample purity is 100%. The mass spectrum for the positive MS peak shows two masses at m/z 218 and 457. The former is the API $[M+H]^+$ and the latter is a sodium-adducted dimer $[2M+Na]^+$.

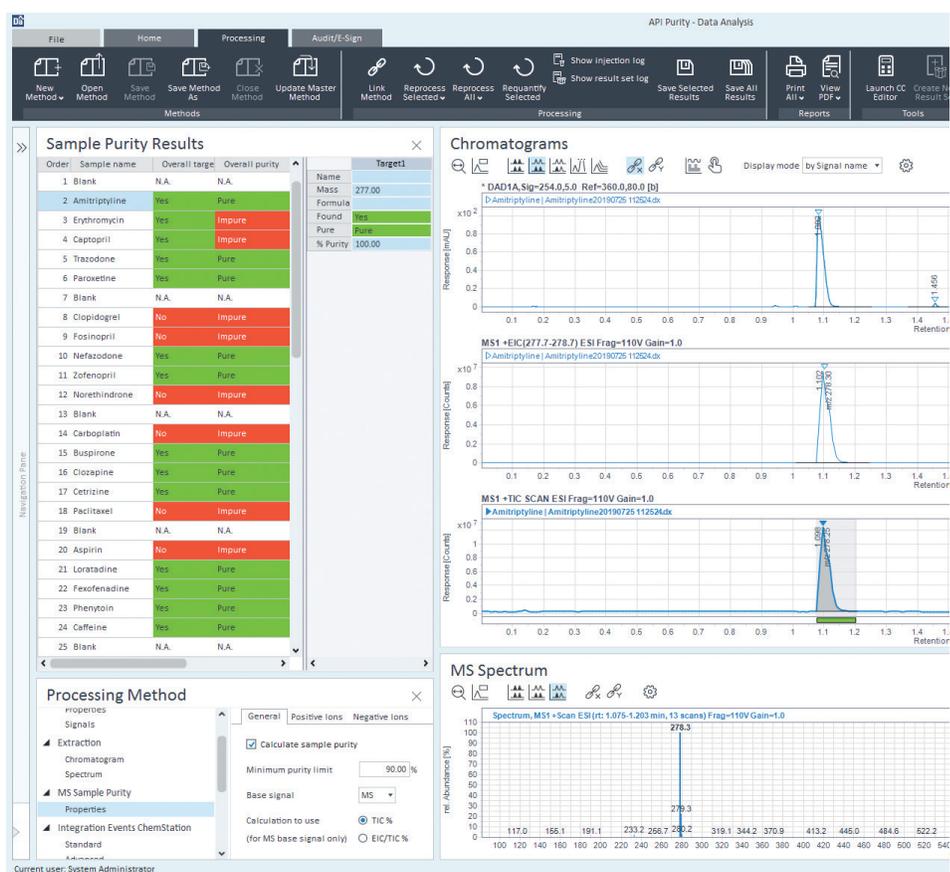


Figure 4. Agilent OpenLab CDS 2.4 data analysis UI for the sample purity workflow. The Sample Purity Results window shows the sample purity results of a sequence of injections. Each row contains the sample name and whether the sample is pure or impure based on the limit set in the processing method (>90% as pure). The UV and MS chromatograms are displayed to the right, with MS spectra of selected peaks shown below.

After three months at room temperature, the MS chromatograms have two peaks, with base peaks at m/z 218/216 and 433/431 for positive/negative scan modes, respectively. m/z 218 corresponds to the $[M+H]^+$ adduct, while m/z 433 is a new substance. The UV peak at 254 nm, shown in Figure 6B, is composed of this new substance at m/z 433, not the target mass of m/z 218. With captopril having a nominal mass of 217 Da, m/z 433 is likely $[2M-2H+H]^+$. When looking at the structure of captopril (Figure 5), it can be deduced that a disulfide bond can be formed over time. In this case, mass information is invaluable to determine the degradation product of the API. With the detection of the disulfide bonded dimer of m/z 433, it can be seen that the sample purity is only 58.49% for captopril.

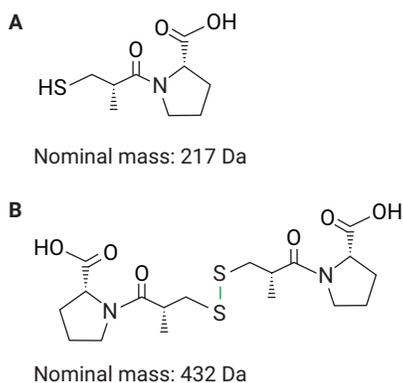


Figure 5. Molecular structure of captopril and the disulfide bound dimer.

Sample Purity Results

Target mass	Target found in analysis	Purity (%)	Purity result
217.0	Yes	100.00	Pure

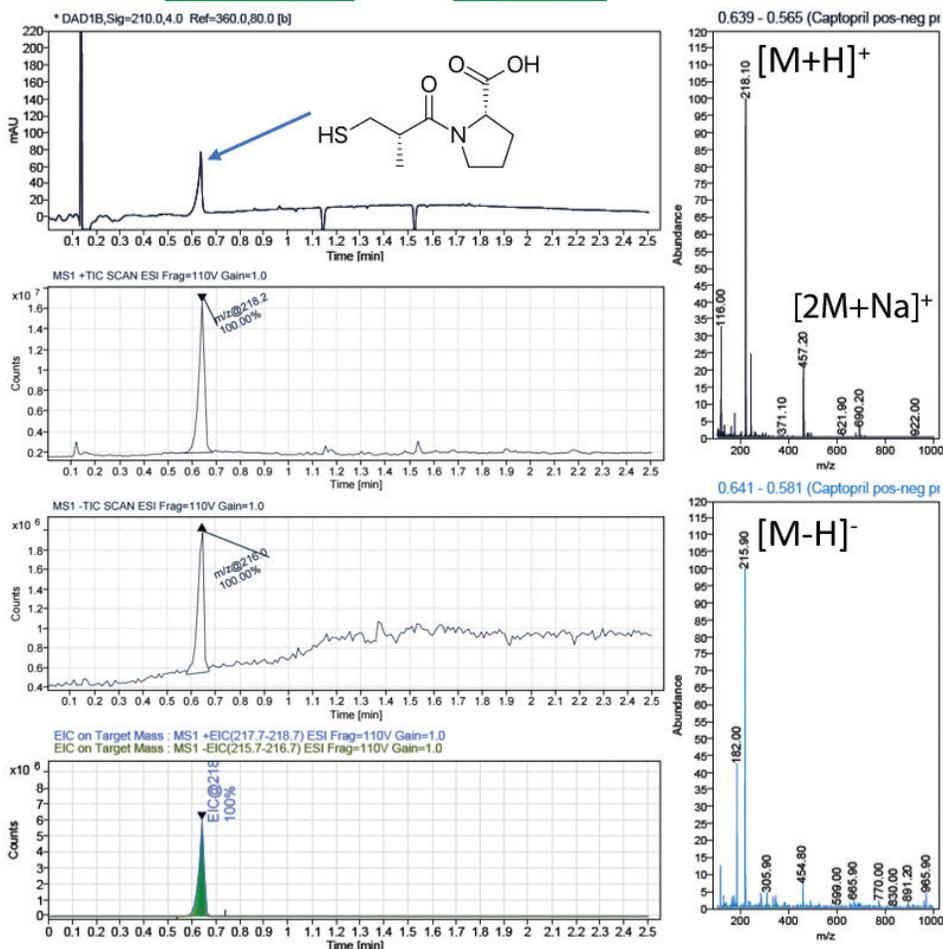


Figure 6A. Freshly prepared captopril. The report includes the sample purity results at the top and the UV and MS chromatograms with the base peak m/z labeled. There is also an EIC of the target compound to show which peaks are comprised of the API. The MS spectra are shown to the right with adduct labels annotated manually for clarification.

Mass information is valuable for identifying impurities of APIs. Having a mass selective detector increases productivity with the capability to discern coeluting analytes. The InfinityLab LC/MSD iQ is the perfect system in combination with UV detection for sample purity workflows by adding mass information to the analysis. This is important when the sample absorbs poorly in the UV or when standards are hard to obtain. MassHunter WalkUp allows multiple users to submit samples through an intuitive user interface that does not require knowledge of mass spectrometry. In combination with an InfinityLab LC/MSD iQ, a true open-access experience is ensured, leading to fast results while saving precious time in labs where decisions must be made quickly.

Sample Purity Results

Target mass	Target found in analysis	Purity (%)	Purity result
217.0	Yes	58.49	Impure

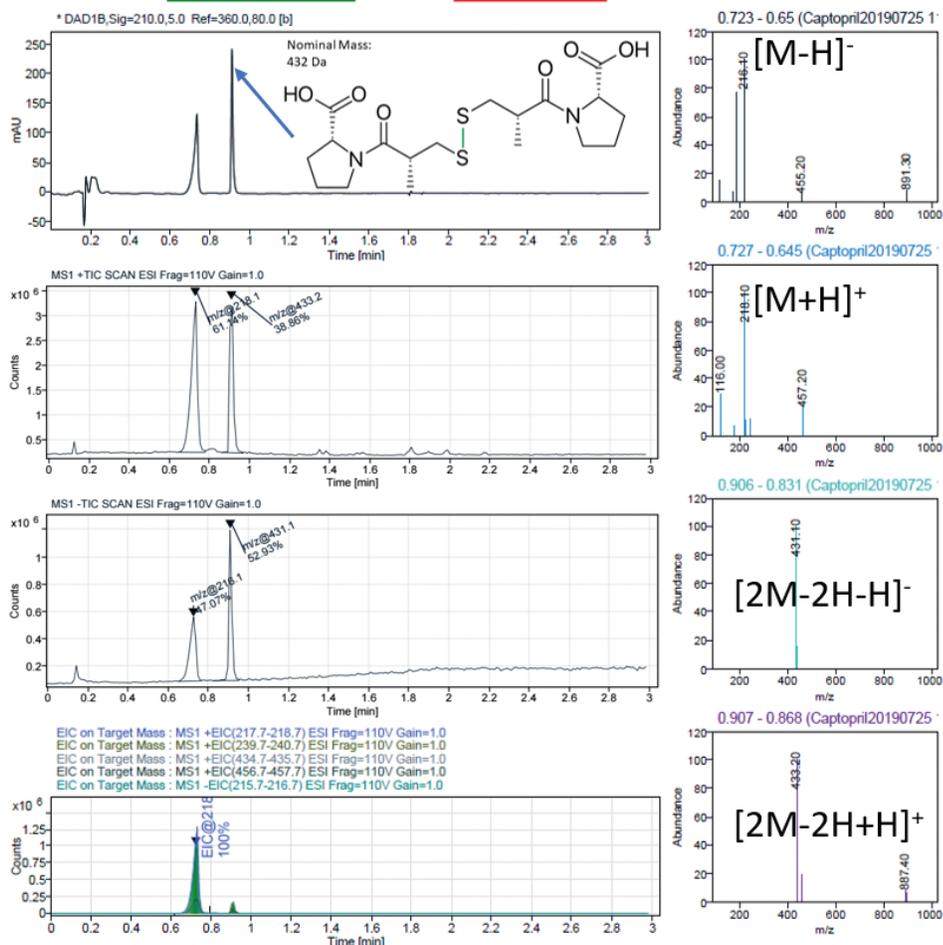


Figure 6B. Captopril sample after three months at room temperature. There is a new substance at 0.9 minutes with m/z 433, which most likely indicates that a sulfide bond has formed between two molecules of the API. With the mass information, this new substance can easily be identified. These reports are automatically generated with WalkUp, and will be emailed directly to the sample submitter. The MS spectra are shown to the right with adduct labels annotated manually for clarification.