Detection of Label Photoinitiator in a Potent Product: A Case Study

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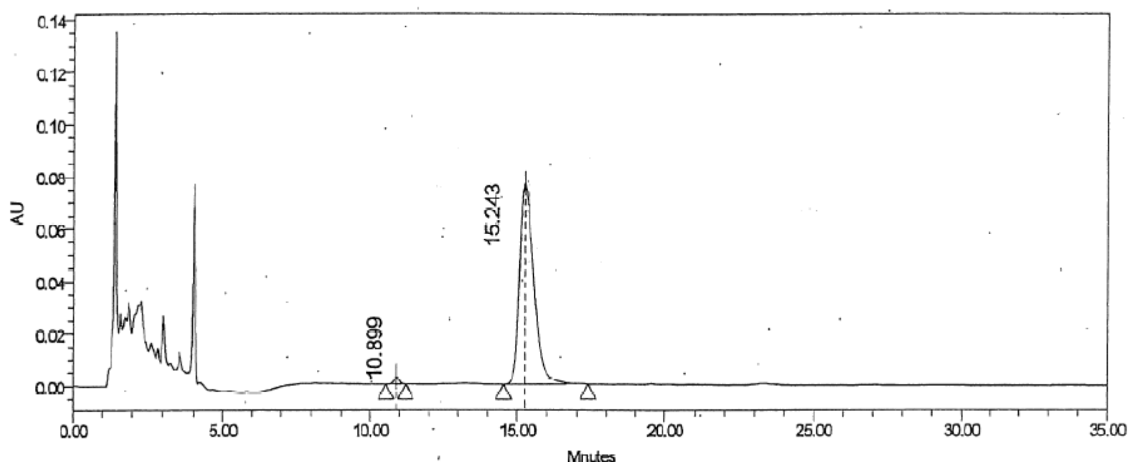
OBJECTIVE

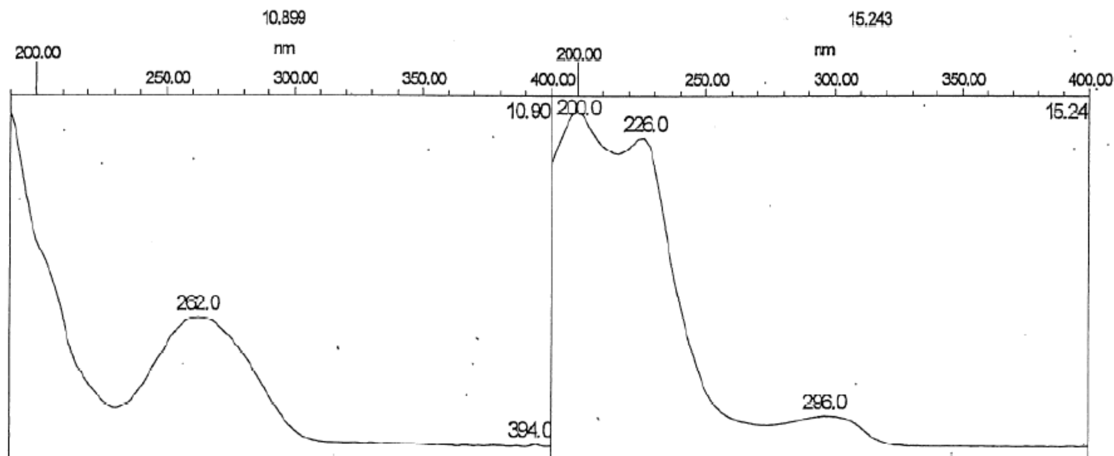
To describe the detection and identification of a benzophenone, used as a bottle label photoinitiator, encountered during routine stability monitoring of a potent drug product utilizing a sensitive analytical method.

BACKGROUND

An unidentified peak (UNID) at RRT 0.72 was determined to be increasing during routine stability monitoring of labeled packaged product with quantifiable amounts appearing at the 3 month time point. In three months stored at 25°C/60% relative humidity, the unknown at RRT 0.72 grew from undetected to approximately 0.5% AUC (area under the curve) with a specification limit of 1.0% AUC for unknown, unidentified peaks. The retention characteristics and spectral scan of this UNID were inconsistent with the API and known degradation products. The sample chromatogram below shows the elution of the unknown peak and the active ingredient, detected at approximately 10.8 and 15 minutes, respectively.



The spectral scan was acquired using a photodiode array, or diode array, detector (DAD) and illustrates the dissimilar spectral scans for the unknown and the active. If the unknown were a degradation product of the active, the spectral scan of the degradant would have a similar absorbance pattern as the active ingredient (see below).



Furthermore, this unknown was not seen during method validation and forced degradation studies nor was it detected in earlier stability studies where the product was stored in unlabeled bottles. It was also confirmed that the unknown was absent in placebo tablets stored on stability in unlabeled bottles.

METHODS

The stability-indicating method employed reverse phase HPLC separation with a Zorbax CN (4.6 x 250 mm, 5 µm) column utilizing UV detection at 225 nm where 1 µg of sample was eluted isocratically with H2O/CH3CN/H3PO4: 60/40/0.05 (v/v/v) at a flow rate of 1.5 mL/minute. Analysis time was 35 minutes with a typical retention time of 15 minutes for the API. The unknown peak eluted at approximately 10.8 minutes with an assigned relative retention time (RRT) of 0.72. This method was successfully run on an Agilent 1100, 1200 and a Shimadzu LC2010.

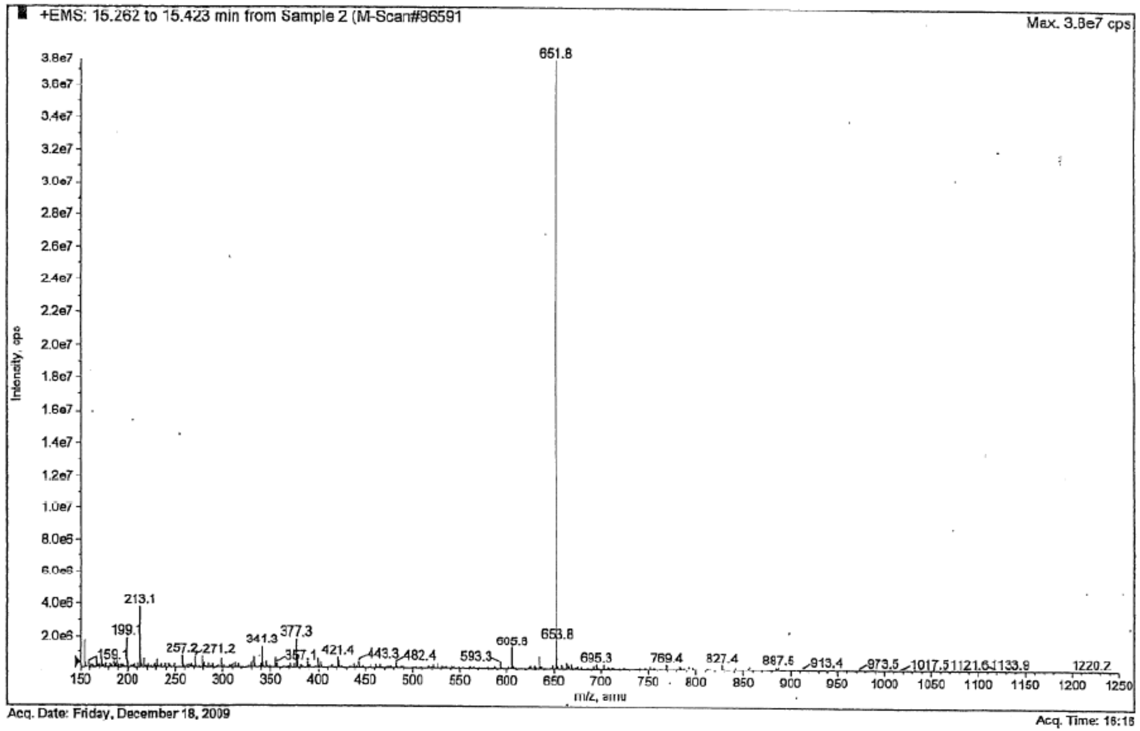
For LC-MS/MS, the mobile phase composition was modified to 0.1% aq. formic acid/0.1% formic acid in CH3CN: 60/40 (v/v) to minimize detection interference. Sequential detection was performed by UV absorbance using DAD followed by positive ion ESI MS/MS using a Sciex 4000 QTRAP MS.

During the course of the LC-MS/MS investigative phase, it was theorized that the unknown may be amenable to GC-MS separation and detection. A GC-MS method was devised using column DB-1 (30 m x 0.32 mm x 0.25 µm). The He flow rate was set to 5 mL/min with an injector temperature of 160°C and a split ratio of 5:1. A temperature program was employed (hold for 7 minutes at 40°C, ramp at 15°C/min to 160°C, ramp at 25°C/min to 260°C, hold for 15 minutes). MS analysis was performed utilizing a Perkin Elmer TurboMass Quadrupole MS, EI+ ion mode.

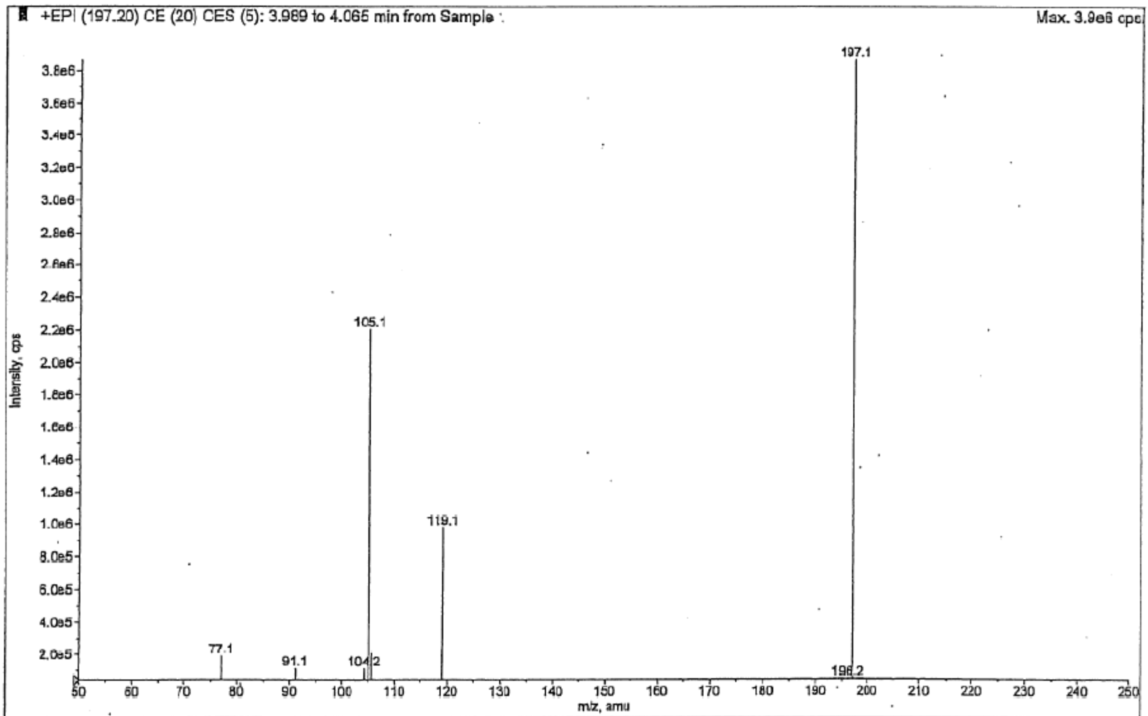
RESULTS

The ESI-MS spectrum for the unknown indicates a major possible (M+H)+ pseudomolecular ion at m/z 197.1; the comparison of the active shows the anticipated (M+H)+ at m/z 651.8. The UNID was further fragmented and yielded cleavage ions at m/z 77, 91, 105 and 119, suggesting a substituted phenolic structure consistent with a benzophenone compound. Spectrum for the active and the unknown are shown below.

MS Spectra for the Active Pharmaceutical Ingredient:

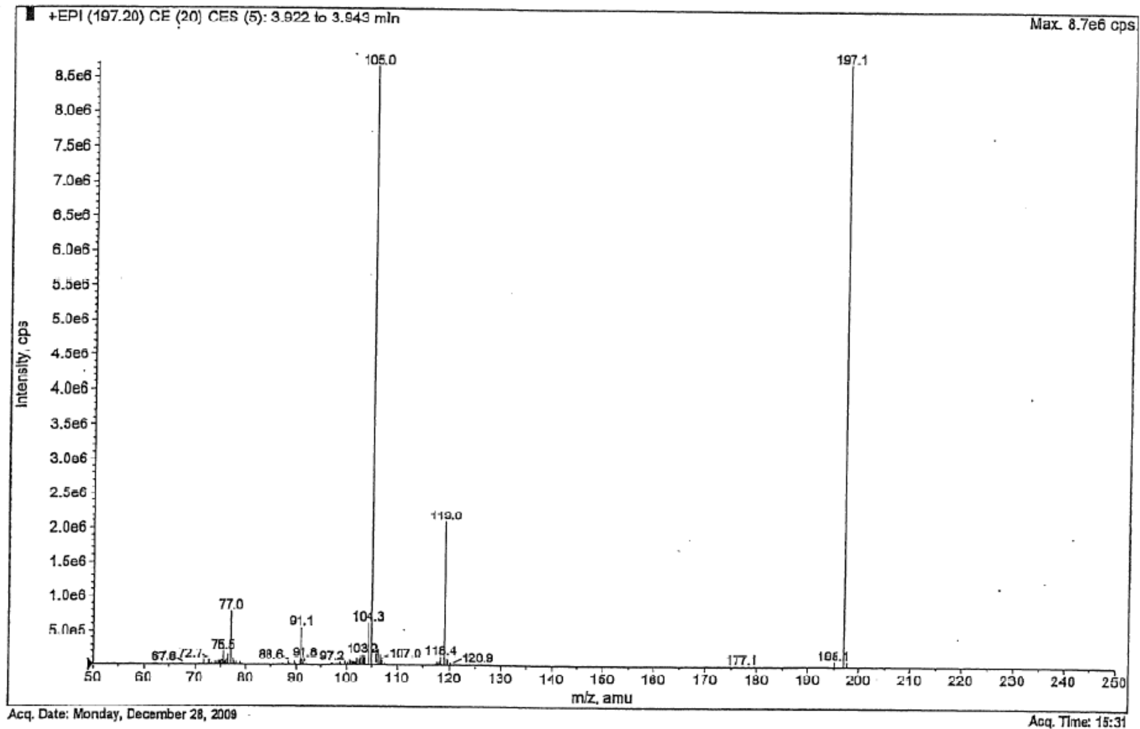


MS Spectra for the UNID at RRT 0.72 displaying cleavage ions:

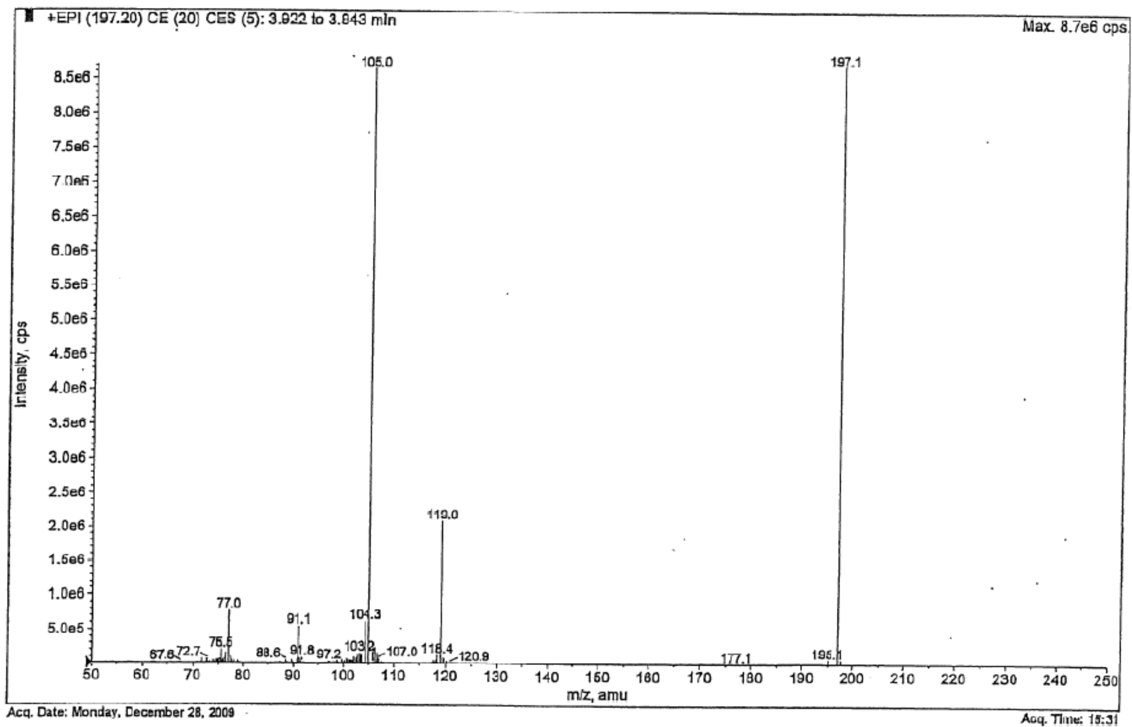


Three benzophenone compounds were determined to be present in the label varnish as photoinitiators: 2-methylbenzophenone, 4-methylbenzophenone and 2,4,6-trimethylbenzophenone. Each of these three benzophenone compounds was present at approximately 7.5% of the varnish composition (22.5% of the total varnish composition for all three benzophenone compounds); no other source of benzophenones was identified in the ink, desiccant or other packaging components. Reference material for the three benzophenone compounds was purchased commercially; the response and spectra of the 2-methyl and 4-methylbenzophenone isomers were consistent with the unknown compound, see below.

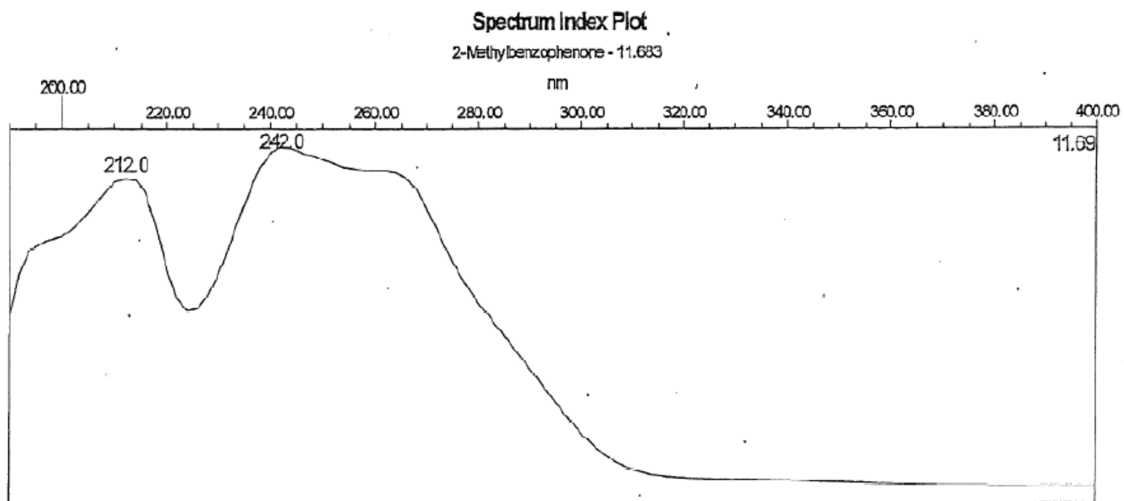
MS Spectra for 2-Methylbenzophenone:

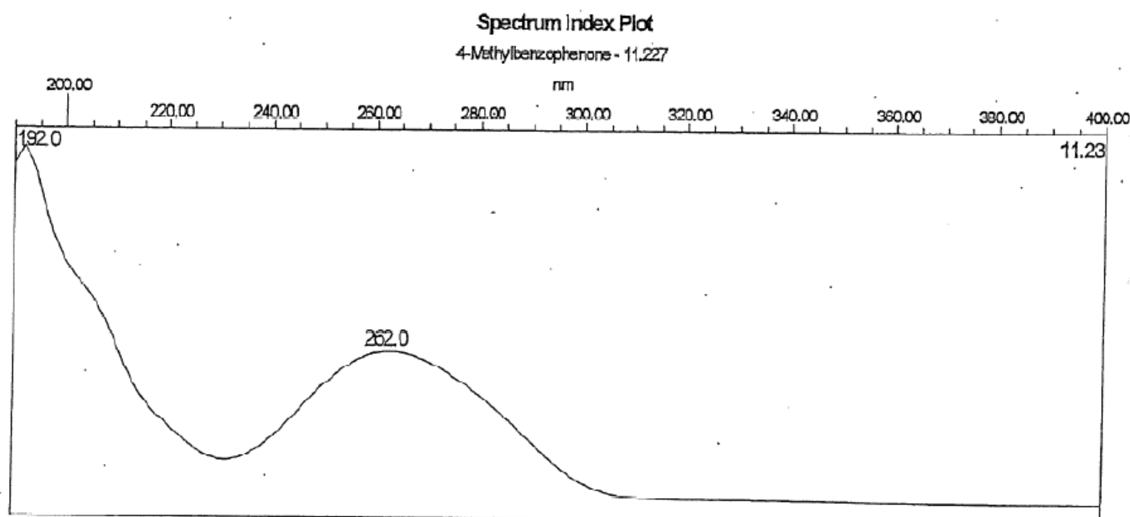


MS Spectra for 4-Methylbenzophenone:



The GC-MS portion of the investigation analyzed a tablet extract which yielded ions similar to those of the unknown in a sample analyzed by LC-MS/MS. The EI spectrum library was researched and 4-methylbenzophenone was identified as the best-fit match. To further verify the identity of the unknown peak, additional laboratory work was performed utilizing DAD detection; the spectra for the 2-methyl and 4-methylbenzophenone isomers were compared to a sample of label extract. Three figures below represent the UV spectra obtained for the three compounds.





DISCUSSION

The use of potent compounds as active pharmaceutical ingredients (APIs) has been on the rise in recent years. This has, in turn, spurned a need for highly sensitive analytical methods that can detect small amounts of API, typically by high pressure liquid chromatography (HPLC). The case under discussion is a product formulated at 5, 25 and 50 µg API in 140 mg tablets, requiring an analytical method that is sensitive enough to quantitate the active in a complicated excipient matrix. See below for product composition, in mg.

Table 1. Composition of Drug Product Formulation

API (µg)	Excipient (mg)	Percent of API
5 µg	139.995 mg	0.004%
25 µg	139.975 mg	0.018%
50 µg	139.950 mg	0.036%

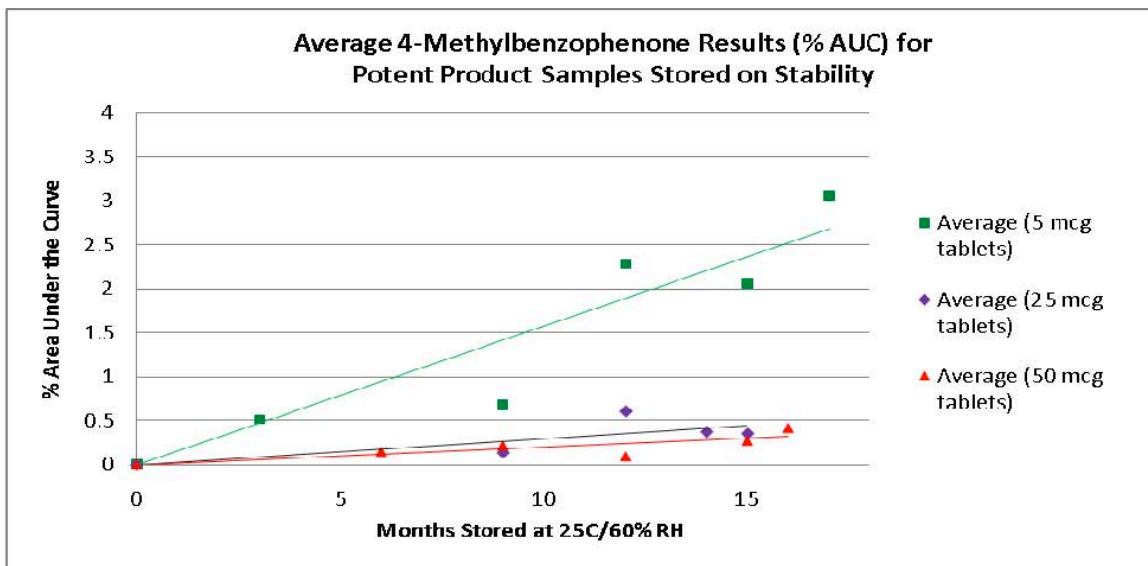
Table 2. Active Pharmaceutical Ingredient and Excipient Load, mg/mL

API (µg)	# Tablets for Sample Preparation	API Concentration	Excipient Load
5 µg	20	0.01 mg/mL	279.99 mg/mL
25 µg	4	0.01 mg/mL	55.99 mg/mL
50 µg	2	0.01 mg/mL	27.99 mg/mL

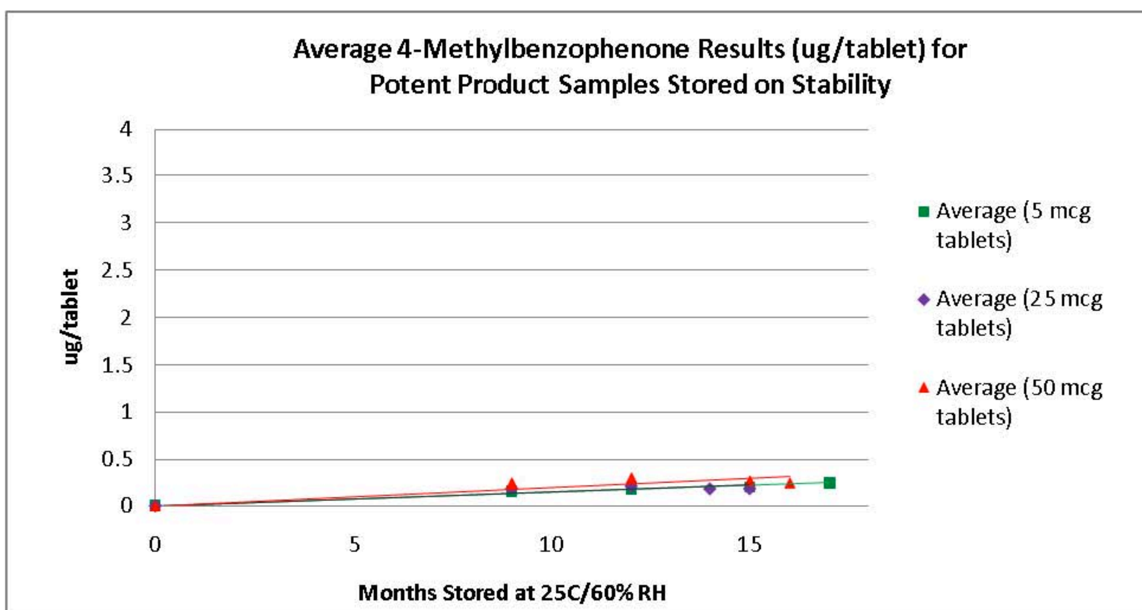
It is the very sensitive nature of this method that lead to the detection of a benzophenone compound within 3 months of CRT (25°C at 60% relative humidity) storage. This benzophenone was identified by MS and DAD spectral comparison with commercially available reference material. The benzophenone compound of interest was listed as an ingredient in the varnish used as a photoinitiator during the manufacture of the drug product's commercial label.

The original analytical method reported degradants and unknown impurities as percent "area under the curve" (% AUC). The % AUC result was determined by integration of all peaks, unrelated to placebo, in each sample chromatograph; the % of each degradant and impurity was then expressed as a percent of the total area integrated. The current analytical method has a validated LOQ of 10 ng/mL (0.01 µg/mL) and the analytical method specified the sample preparation as 100 µg API dissolved in 10 mL of diluent.

While stored under CRT conditions for 3 months, the benzophenone component of the label varnish diffused through the bottle wall to adulterate the product contained within to a level detectable by the analytical method. Due to the sample preparation scheme and reporting convention defined by the validated method, the levels of benzophenone were much higher in the 5g formulation in comparison with the 25 µg and 50 µg formulations. As shown below, the level of benzophenone continues to increase over time while the product remains stored on stability. When calculated on the % AUC basis, the 4-methylbenzophenone result is artificially inflated in the 5 µg formulation due to the greater number of tablets used in the preparation of samples for analysis (see below).



Once the unknown compound was identified as 4-methylbenzophenone, a method to quantify this compound on a µg/tablet basis was developed and validated using commercially available reference material. The levels of 4-methylbenzophenone continue to be monitored on stability until the affected product batches reach the end of the stability monitoring period. See graph below.



CONCLUSION
A method for the determination of 4-methylbenzophenone has been developed and the product continues to be monitored on stability. Through approximately 18 months of stability monitoring, the levels of benzophenone remain below 0.375 µg per tablet (<0.0003% w/w).

The presence of 4-methylbenzophenone during tablet analysis confirms the unexpected migration of very low levels from the bottle label into the product. If the active were not a potent pharmaceutical agent, it is probable that the levels of 4-methylbenzophenone present in the product would not be detected during the course of routine stability monitoring.