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Strategy for identification of leachables in packaged pharmaceutical liquid formulations

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Abstract

Drug stability is one of the key properties to be monitored in pharmaceutical drug development. Drug degradation products, impurities and/or leachables from the drug product and packages may have significant impacts on drug efficacy, safety profile and storage conditions. In the registration stability samples of an ophthalmic pharmaceutical drug product, an unknown compound was found at a level of 0.19% by HPLC analysis. Subsequent liquid chromatography/mass spectrometry (LC/MS) analysis with electrospray ionization (ESI) indicated that the unknown was not related to the drug substance and was most likely a leachable. Identification of this unknown leachable was needed to evaluate the impact on drug safety. Through systematic extraction of various components or component combination of the packaging materials, and subsequently LC/MS analysis, the unknown was found to be a leachable coming from the varnish applied to the label. In general, using LC/MS alone is not sufficient to elucidate the structure of a complete unknown. Gas chromatography/mass spectrometry (GC/MS) was then conducted with a chemical ionization (CI) source to determine the retention time and mass of the compound of interest. Both CI and ESI sources generated the same protonated molecular ion [M + H] and similar fragmentation ions, which provides a good correlation of the unknown eluted in the liquid chromatogram and in the gas chromatogram. GC/MS with electron impact (EI) was then conducted to obtain the EI mass spectrum of this unknown. It was identified as monomethyl derivative of mephenesin through the NIST library search.

The identification strategy utilized electrospray LC/MS and GC/MS with chemical and electron ionization sources which provided complimentary information for structure elucidation of this unknown compound. This combination approach in conjunction with systematic extraction was necessary for the determination of the source of this unknown in the pharmaceutical drug stability studies.
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Keywords: LC/MS/MS; GC/MS; Leachable identification; Extraction

1. Introduction

Hyphenated analytical techniques in which a chromatographic separation is coupled online with one or more information-rich detectors, such as liquid chromatography/mass spectrometry (LC/MS), gas chromatography/mass spectrometry (GC/MS) and liquid chromatography nuclear magnetic resonance (LC/NMR), have quickly become powerful tools for the identification or confirmation of low or trace level impurities. These techniques have complementary selectivity that require analysis by all to completely define an unknown molecular structure. The LC/MS technique has been widely used in the pharmaceutical industries due to its high sensitivity, selectivity, dynamic range and ruggedness [1–5]. The technique has excellent sensitivity for the detection of trace level impurities and degradation products observed in pharmaceutical drug development and manufacturing process. Mass spectrometry techniques are commonly used to identify leachables in a variety of pharmaceutical products. A leachable from adhesives used in pharmaceutical products was identified using data-dependant LC/tandem mass spectrometry [6]. A leachable from rubber closure was identified by HPLC, UV and MS detectors [7]. GC/MS was also used to study leachables from disposable syringes [8]. However, in some complicated situations LC/MS technique by itself cannot come up with the final structures. In these cases, a strategy that combines LC/MS and GC/MS with NIST library search can provide additional information for complete structural elucidation of an unknown.

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This investigation involved the identification of an unknown leachable found in a registration stability sample of an ophthalmic solution stored in a semi-permeable low density polyethylene (LDPE) bottle. Initial LC/MS/MS analysis of the unknown indicated that the unknown was not structurally related to the active ingredient. Hence, the unknown was identified as a leachable from plastics or labels on the semi-permeable bottles. All packaging for human use must be suitable per FDA guidance on packaging for human drugs [9,10]. The unknown impurity was detected in the final market container when the product label was changed shortly before the start of registration stability program. After 6 months at 40 °C/20%RH test station, an unknown impurity was found above the identification threshold of 0.1% from two separate samples. An investigation was initiated to determine both the source of the unknown impurity and its identification followed by an assessment of the maximum daily exposure. Controlled extraction studies were conducted to determine the source of this impurity using reverse phase HPLC gradient. LC/MS/MS and GC/MS experiments were performed to determine the identity of this unknown in the controlled extraction samples of the all packaging components and in the stability samples.

2. Experimental

2.1. Chemicals and reagents

Chemicals and reagents used in this study are listed below:

Methanol (HPLC-grade, Fisher, Fair Lawn, NJ, USA).
Acetonitrile (HPLC-grade, Fisher, Fair Lawn, NJ, USA).
Trifluoroacetic acid (ACS-grade, Sigma–Aldrich, St. Louis, MO, USA).
Ethyl acetate (ACS-grade, Sigma–Aldrich, St. Louis, MO, USA).

2.2. Liquid chromatography tandem mass spectrometry instrumentation and condition

2.2.1. Mass spectrometry

All LC/MS mass spectral data were collected using an LCQ^{Deca} Ion Trap Mass Spectrometer (ThermoElectron, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. The mass range acquired was from 100 to 800 amu in a positive ion mode. ESI conditions: capillary temperature 300 °C, capillary voltage 3 V, sheath gas 80 (arbitrary units), auxiliary gas 20 (arbitrary units).

2.2.2. Liquid chromatography

A 2690 Alliance HPLC system (Waters, Milford, MA, USA) was used in this study. The separation was achieved using a Waters Symmetry^{TM} C18, 4.6 mm × 150 mm column with particle size of 3.5 μm (Waters, Milford, USA). Mobile phases A and B contained water and acetonitrile in a volume ratio of 90:10 and 10:90, respectively. Both mobile phases also contained 0.05% trifluoroacetic acid. The following HPLC gradient program was applied at a flow rate of 1.0 ml/min: 10% B at 0 min, increased to 100% B at 30.0 min, changed to 10% B at 30.1 min and held at 10% B for 10 min before injection of next sample; column temperature 30 °C; injection size ranged from 100 to 250 μl, sample solvent was water–acetonitrile in a volume ratio of 60:40. A UV6000 LP PDA diode array detector (ThermoElectron, San Jose, CA, USA) was used to monitor the UV–vis signals at either 220 nm or 190–500 nm range.

2.3. Gas chromatography tandem mass spectrometry instrumentation and condition

The gas chromatograph utilized was TRACE GC-2000 (ThermoElectron, San Jose, CA, USA). The capillary column used was a HP-5MS, 30 m × 0.25 mm i.d. × 0.1 μm film thickness (Agilent Technologies, Wilmington, DE, USA). The carrier gas was high purity helium (99.999%, Welding Supply Co., Inc., NJ, USA) under a constant column flow rate of 1 ml/min. A gas purifier (VICI, Fisher, PN 05730-2) and a moisture trap (VICI, Fisher, PN 05-730-9) were connected in series on the helium line to remove hydrocarbon impurities and trace water in the helium gas. The oven column temperature was initiated at 40 °C, held for 1 min, raised to 200 °C at 10 °C/min, and held at that temperature for 5 min. The injector temperature was 320 °C and the injector was operated in the splitless mode with 1 μl injection. The purge flow was set at 50 ml/min and the purge time was set at 1 min. The GC/MS interface temperature was 300 °C. DSQ single quadruple mass spectrometer (ThermoElectron, San Jose, CA, USA) was equipped with both electron impact and chemical ionization sources. The EI mode operated with ionization energy of 70 eV was used to acquire EI mass spectrum for structure elucidation. Chemical ionization source with methane (UN1971, Ultra high purity, AGL Welding Supply Co., Inc., NJ, USA) was operated at a flow rate of 2 ml/min to determine the protonated molecular ion [M + H] for the compound of interest. The mass spectral scans were carried out continuously from 50 to 500 amu during GC analyses with an ion source temperature at 220 °C.

2.4. Sample preparation

The following sample and packaging components were extracted and/or analyzed:

drug stability sample (40 °C/20% relative humidity, 6 months);
LDPE bottle, plug and cap;
label with ink and varnish;
label: Avery GEXPBE (rainbow);
varnish: EC001245 Film III 16Y5;
label ink (Orange): QY001645 Film III 16Y5.

Controlled extraction studies were performed to investigate the source of the substance in the packaging materials which could be leached into the drug product solution. The packaging components in various combinations were extracted by a 50 ml mixture of water and methanol (1:1). The extrac-

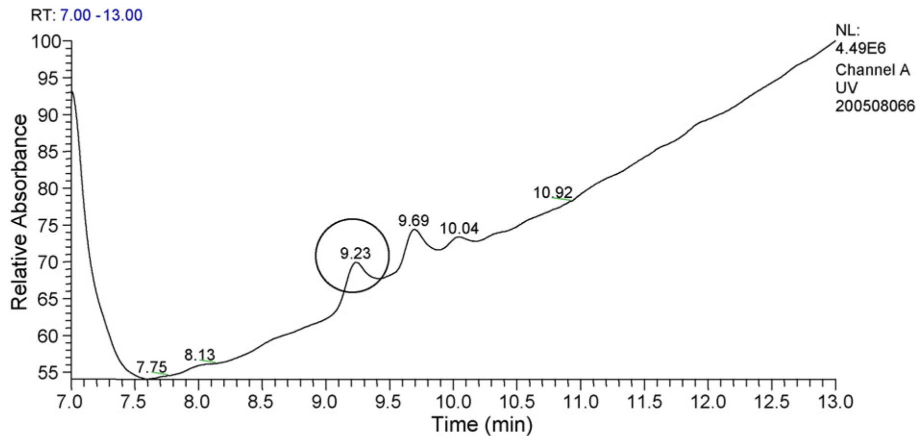


Fig. 1. HPLC chromatogram of the stability sample solution stored at 40 °C/20%RH for 6 months.

tion solution was heated in an oven at 50 °C for 3 days. The methanol used in the extraction was then removed using a TurboVap II (Zymark, Hopkinton, MA, USA). Ethyl acetate was then added to extract trace organic components from the aqueous portion. The collected organic portion was evaporated using a TurboVap. The residues were dissolved in about 0.5 ml of methanol prior to LC/MS and GC/MS analyses.

3. Results and discussion

3.1. Liquid chromatography/mass spectrometry

Fig. 1 shows the chromatogram of a drug stability sample stored at 40 °C and 20% relative humidity for 6 months. An unknown peak was found at 9.2 min in the chromatogram and the unknown peak had a protonated molecular ion [M + H] of

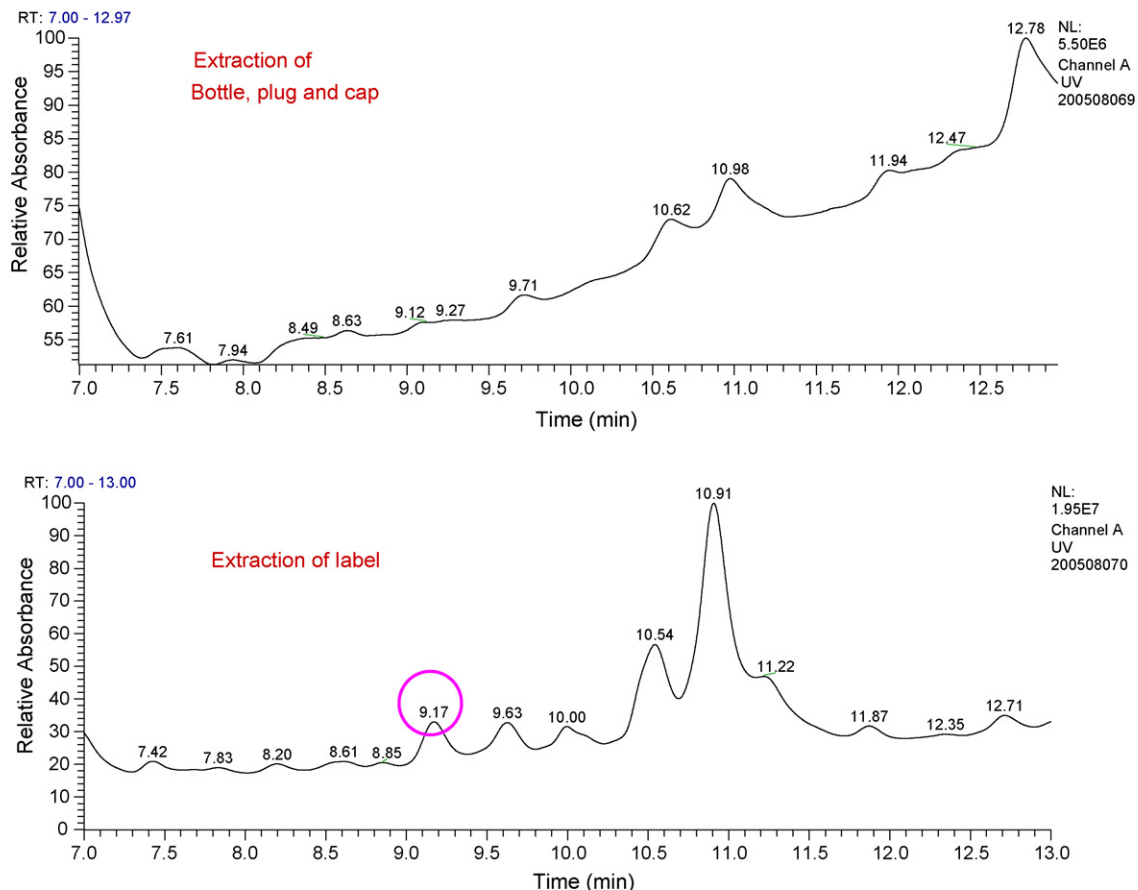


Fig. 2. HPLC chromatograms of two extractions: (Top) extraction of bottle, plug and cap; (Bottom): extraction of label.

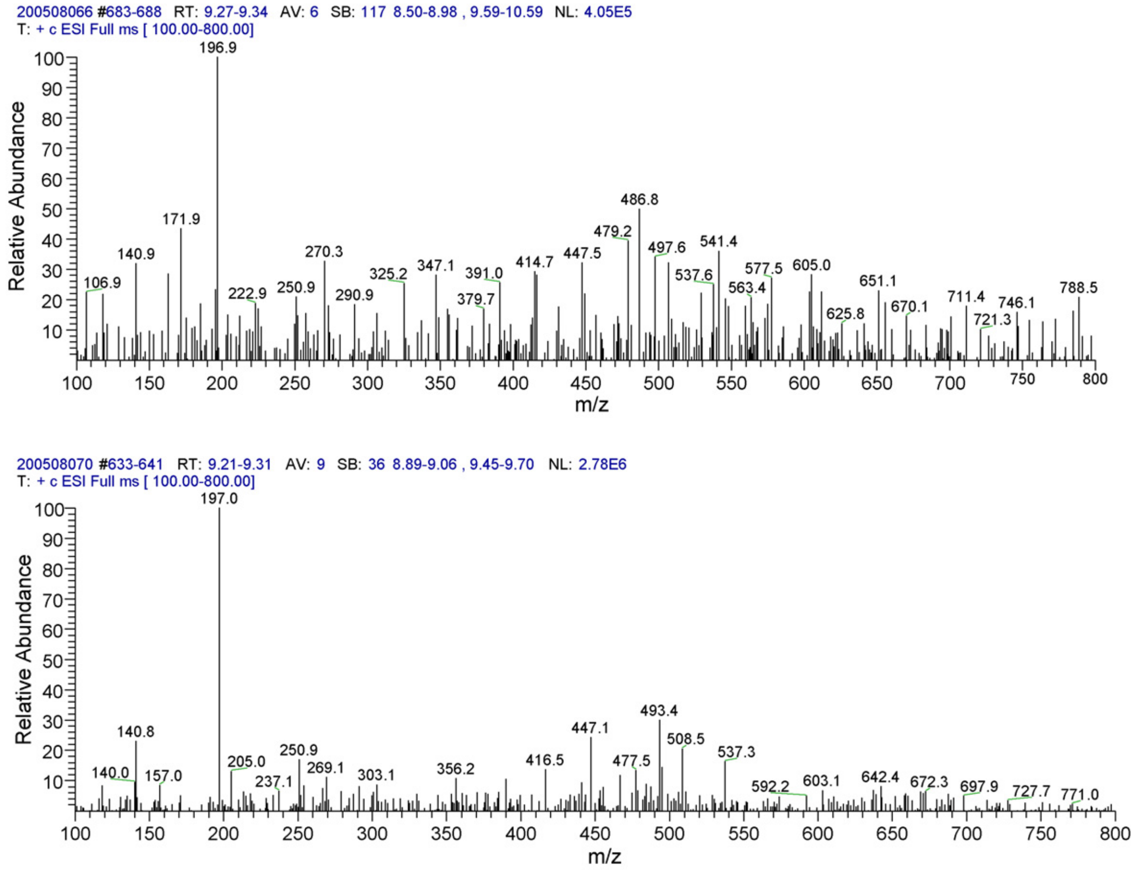


Fig. 3. Mass spectra of the peaks at 9.2 min for (Top): stability sample; (Bottom): extraction of label.

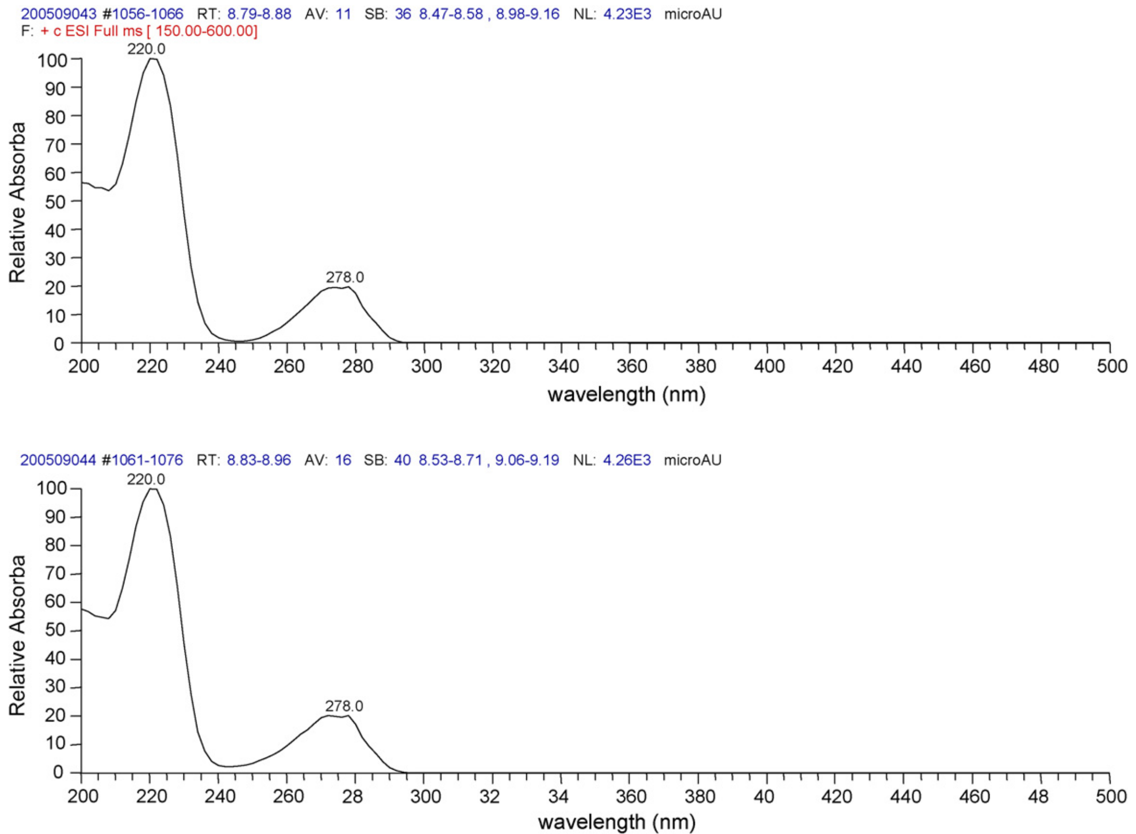


Fig. 4. UV–vis spectra of the peaks at 9.2 min for (Top): stability sample; (Bottom): extraction of label.

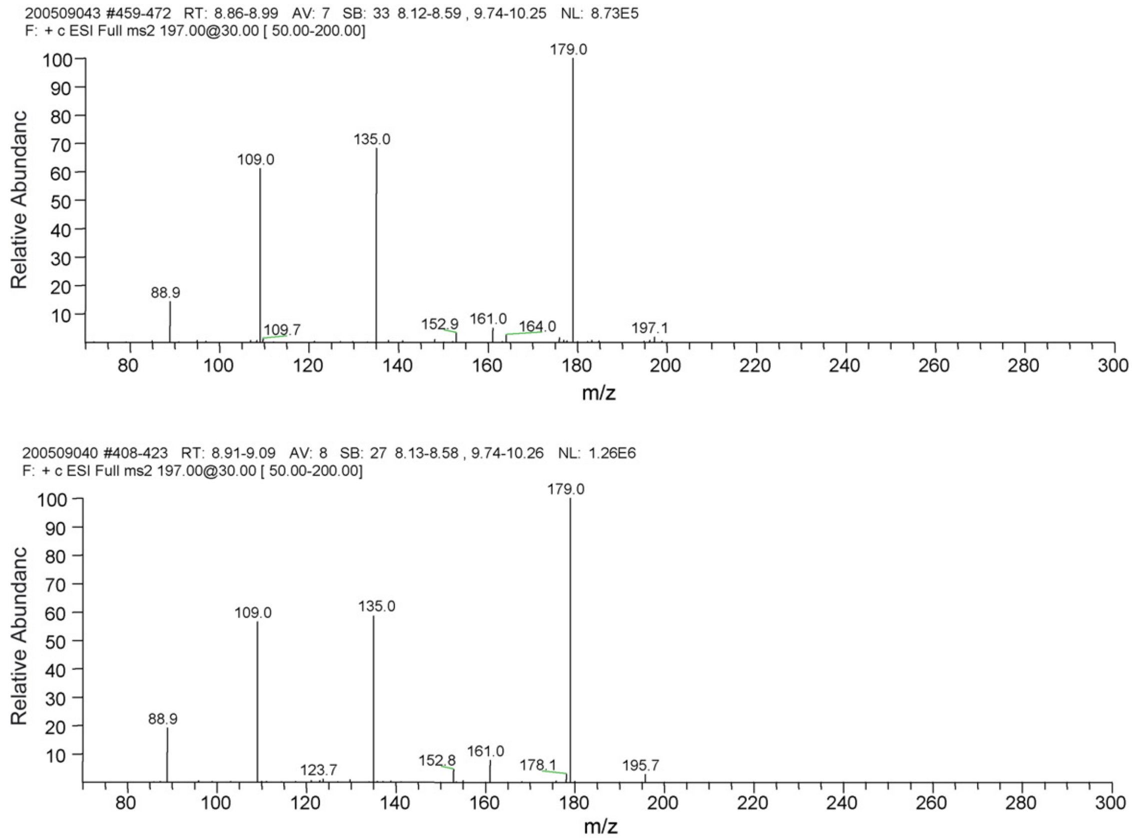


Fig. 5. LC/MS/MS mass spectra of the peaks at 9.2 min for (Top): stability sample; (Bottom): varnish extraction.

197. Based on the mass spectrum and the UV–vis spectrum, this unknown compound is not structurally related to the drug substance. The unknown leachable was confirmed in two separate samples at the same condition by the HPLC method. In addition, the unknown leachable from the label extraction procedure was confirmed against the ophthalmic stability solution which contained at the contents of 5 separate bottles. In order to understand the source of the unknown, a systematic solvent extraction

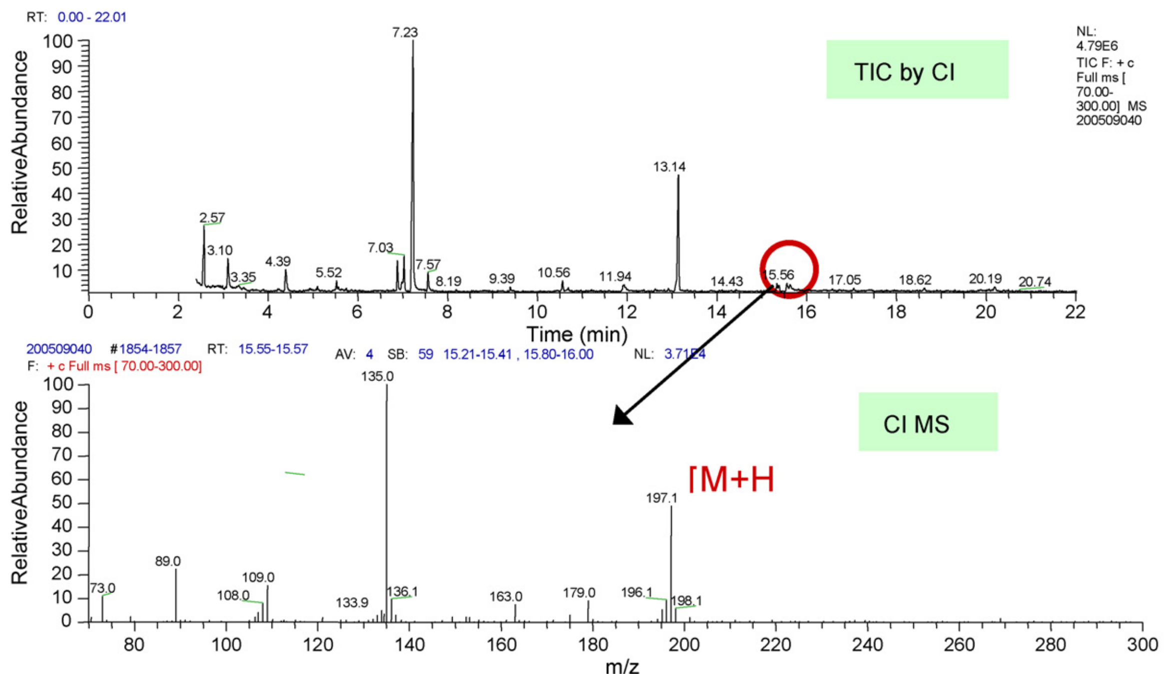


Fig. 6. (Top): Total ion chromatogram of varnish extraction detected by GC/MS with chemical ionization (CI) mass spectrometry; (Bottom): CI mass spectrum of the peak at 15.6 min.

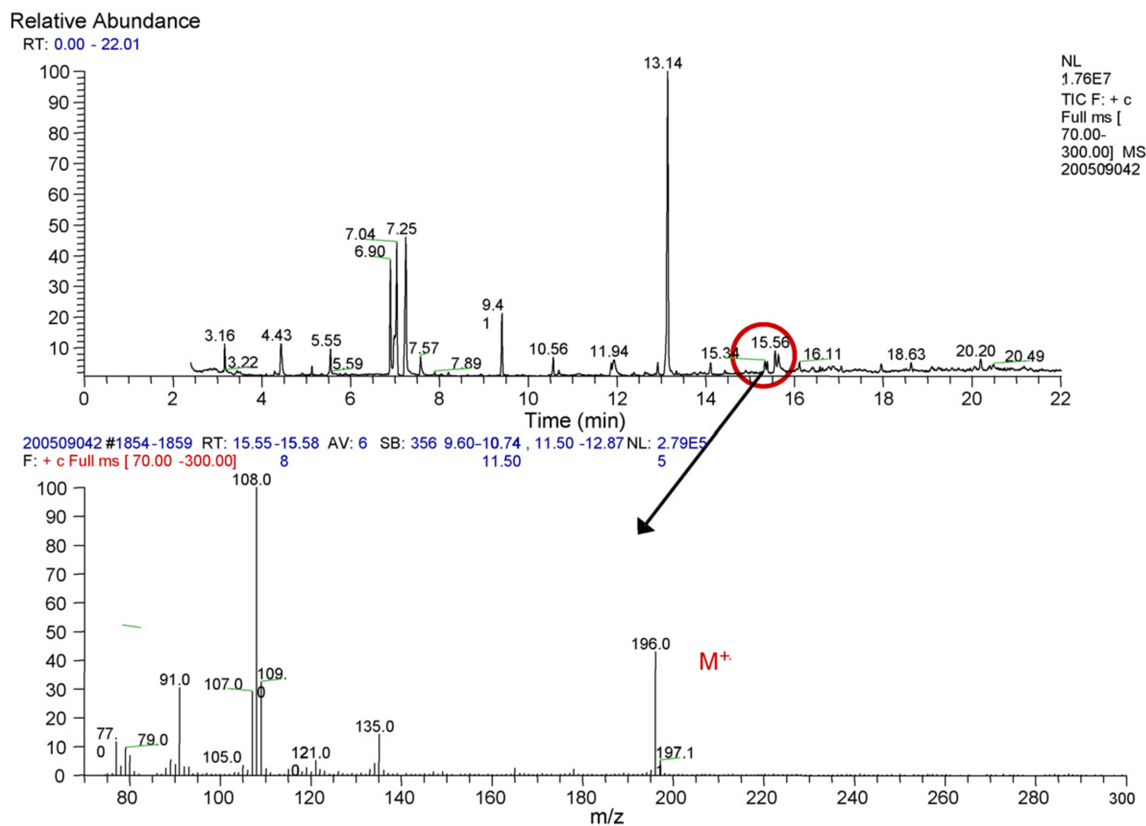


Fig. 7. (Top): Total ion chromatograms of Varnish extraction detected by GC/MS with electron impact (EI) mass spectrometry; (Bottom): EI mass spectrum of the peak at 15.6 min.

of various packaging components was conducted using a mixture of water and methanol in a ratio of 1:1. One extraction was performed on the combined polyethylene bottle, plug and cap, but no label. The other extraction was performed on the bottle label (rainbow label) only. Fig. 2 indicates that the extraction solution of bottle, plug and cap does not have a clear peak at around 9.2 min in the chromatogram. The retention time might vary since samples were measured at different dates during several weeks of investigation. The retention time deviation of 0.06 min (9.23–9.17 = 0.06 min) is acceptable in LC analysis.

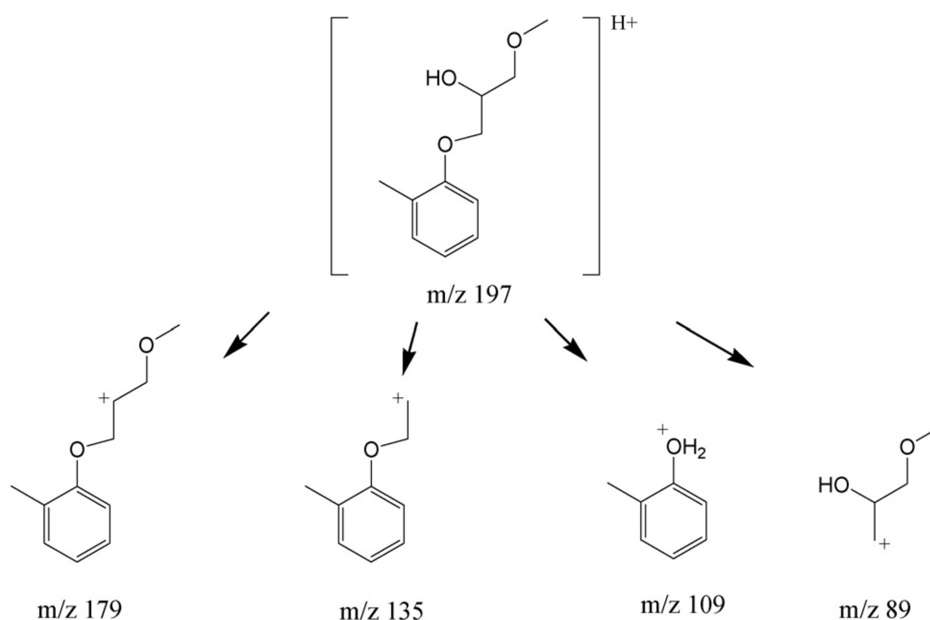


Fig. 8. Proposed ion fragmentation pathway and fragment ion substructures.

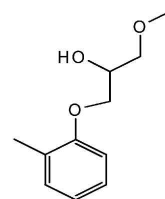
A small "peak" at 9.27 min appeared only in an extraction solution, Fig. 2. However, LC/MS results indicate that this is not the peak of interest based upon both retention time and mass spectral result. If a peak in an extract has the same retention time as the unknown but does not have the "right" mass, the peak is discarded as not being the unknown peak of interest. In contrast, the unknown peak observed in the stability sample also appeared in the chromatogram of the label extraction solution. The compound that eluted at around 9.2 min in the chromatogram of the label extraction is the same unknown compound found in the stability sample since they have the same retention time, similar mass spectra with an [M + H] ion of 197, and have similar UV spectra. See Figs. 3 and 4. The unknown has a molecular weight of 196 Da. The results confirm the unknown was leaching from the bottle label.

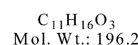
As the bottle label contains label adhesive, label ink, and varnish, further experiments were conducted to investigate the adhesive, ink, and varnish separately. No apparent peak was found in the extraction of label adhesive paper, indicating that the unknown was not coming from the adhesive. Further experiments were conducted to investigate the label ink and varnish separately. One drop of the label ink and one drop of varnish were dissolved in methanol to form solutions prior to LC/MS analysis. Both sample solutions showed the peaks at the same retention time as the unknown peak in the stability sample, but their UV spectra were different. The varnish matched the MS spectrum of the unknown in the stability sample while the orange ink did not match the unknown spectrum. Fig. 5 shows the MS/MS spectra of the protonated molecular ion (*m*/*z* 197) for the compound eluting at 9.2 min in the stability sample and in the varnish solution. The ion fragmentation patterns match well in these two samples, indicating that the component in the varnish solution is the same compound found in the stability sample. The resulting fragmentation pattern could be also used as a reference in the GC/MS chemical ionization mass spectral analysis (see discussion in the next section). The summary of the LC/MS results indicate that the unknown in the stability samples came from the varnish used coat the labels.

3.2. Gas chromatography/mass spectrometry

Although LC/MS provides the molecular weight and fragmentation information, it was still not enough for structure elucidation. In order to obtain further molecular information, GC/MS with chemical ionization was used to detect the compound with the same protonated molecular ion [M + H] of *m*/*z* 197 as the unknown in the stability samples. As shown in Fig. 6, GC/MS studies reveal that the compound eluting at 15.6 min in the gas chromatogram has an [M + H] ion of 197. The compound has several unique fragments (*m*/*z* 89, 109, 135, 179) in the CI mass spectrum, Fig. 6. These fragments were also observed in the MS/MS spectrum of the unknown during LC/MS studies, Fig. 5. The same protonated molecular ion [M + H] and the similar fragmentation ions between GC/MS and LC/MS indicate that the peak observed at 15.6 min in the gas chromatogram corresponds to the unknown peak observed at 9.2 min in the liquid chromatogram. Even though chemical ionization in the gas phase and electrospray ionization in the liquid phase are two different ionization processes, they both generate the same protonated molecular ions [M + H]. These identical protonated molecular ions established a peak correlation between gas chromatograms and liquid chromatograms.

Based on chemical ionization results, election impact ionization was used to generate the EI mass fragmentation of the peak at 15.6 min. The EI mass fragmentation results from the compound could be identified by the NIST Library (Version 2) search. Fig. 7 shows the EI mass spectral data, where the peak eluting at 15. 6 min has an "odd electron" ion at *m*/*z* 196 (a radical $M^{\bullet+}$ ion, not a protonated [M + H] ion). This observation further confirms that the compound eluting at 15.6 min in the gas chromatogram has a molecular weight of 196 Da. The above EI and CI mass spectral data are fully complementary to each other in the molecular weight determination. Based on the EI mass spectrum obtained, the unknown in the stability samples was identified as monomethyl derivative of mephenesin through the NIST library search with a good matching factor of RSI 807. Its structure is shown below:





1-Methoxy-3-*o*-tolyloxy-propan-2-ol

The above structure is consistent with the fragmentation pattern observed in the LC/MS/MS spectra in Fig. 5. The compound has a neutral loss of water (197 → 179), indicating that one hydroxyl group is present in the structure. The substructures of this molecule matches well with the fragmentation pathway, as illustrated in Fig. 8.

4. Conclusion

The combination of LC/MS/MS and GC/MS was applied to the investigation and identification of an unknown leachable in an ophthalmic solution stability sample (40 °C/20% relative humidity for 6 months). This combination approach for unknown identification avoided peak isolation, which could be very time-consuming and labor-intensive for this low level of impurity. In addition, the use of GC/MS with CI provided the ability to identify the same unknown compound observed in LC/MS while GC/MS with EI provided straight-forward structural identification.

This approach in conjunction with a systematic data analyses provided the following conclusions:

(1) the unknown was most likely a leachable as it was not related to the drug substance based on LC/MS/MS results;
(2) the exact source of the unknown was identified by LC/MS analysis as the varnish through systematic extraction of each packaging component;

(3) CI GC/MS provided the information to identify the retention time of the unknown in the GC chromatogram through correlation of the unknown's mass with the mass observed in the LC/MS analysis;
(4) the unknown was identified by comparing its EI mass spectrum with a standard reference spectrum in the NIST database;
(5) the substructures of this compound were consistent with its ion fragmentation pattern observed in the LC/MS/MS;
(6) thus the identified compound in the varnish penetrates the label ink, label, label adhesive, and polyethylene bottle to reach the sample solution during storage.

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