Poster Reprint

ASMS 2020
TP 588

Screening and Identification of extractables in drug container by high-resolution accurate mass LC-MS/MS operated in polarity switching mode

Prasanth Joseph[1]; Saikat Banerjee[1]; Samir Vyas[1]
[1]Agilent Technologies, BENGALURU, India

Introduction

Drug containers meant to protect the drug from environmental contaminants. However, they themselves become a source of contamination. Major sources of extractables are polymers, elastomers, vulcanizing agents, dyes and adhesives.

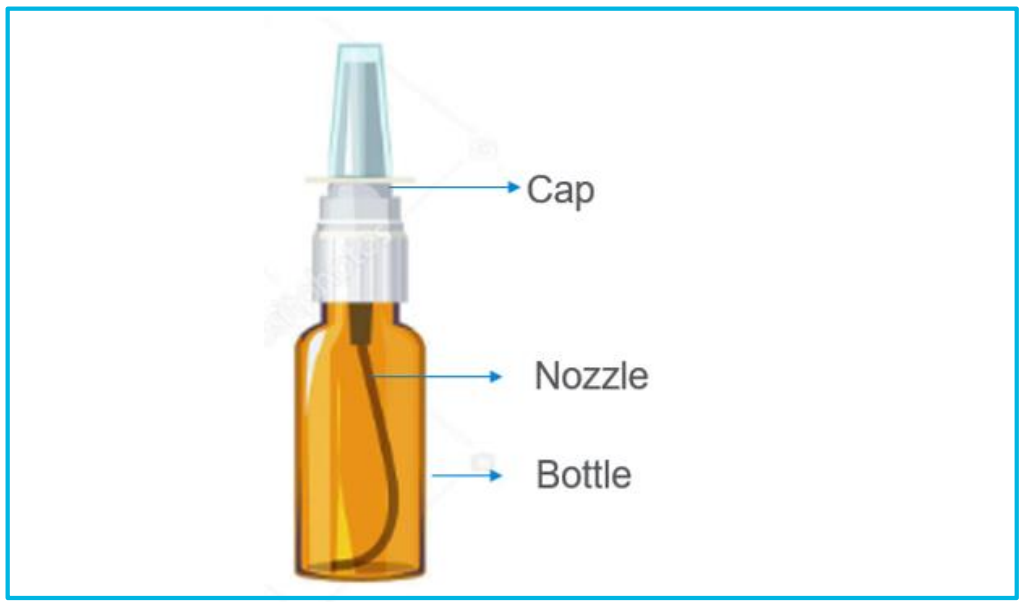

Figure 1. Drug container closure system

High-resolution accurate mass LC-MS/MS can be effectively used to identify and confirm these compounds. Both targeted and untargeted workflows are employed for screening and confirmation of known unknowns. LC-QTOF system installed with ESI source operated in polarity switching mode increases the throughput of this analysis. A targeted approach is based on the screening of the identified *m/z* species and their isotopic patterns against an accurate mass database. Compounds which are not a part of the database can be selectively picked for MS/MS analysis and identification would be based on fragmentation pattern.

Figure 2. 1290 Infinity II coupled to 6545 LC/Q-TOF

Experimental

Sample Preparation

Samples were prepared as per the Parenteral and Ophthalmic Drug Products (PODP) extraction matrix. A medicine bottle was purchased from a local store. Components such as bottle, rubber cap, and nozzle were separated. Sonication of these parts for 1 hour with solvents such as IPA: Water, aqueous systems with pH 2.5 & pH 9.5 was used for extraction. The resulting clear solutions were directly injected into an LC-ESI QTOF system for analysis. A reverse-phase gradient was utilized for the separation. Extraction solvents used were taken as control samples. Fast polarity switching in TOF MS mode of mass range 50-1300 Da followed by TOF MS/MS acquisition was performed. Fold change analysis between sample and control was carried out in statistical software.

Chromatographic conditions

Mobile phase A: 10 mM ammonium acetate
Mobile phase B: Methanol
Column: Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 × 100 mm, 1.8 µm
Column Temp: 40 deg. C
Injection volume: 10µl

time [min]	%B
0	40
8	100
11	100
11.1	40
Post time 1.5 min	

MS Acquisition parameters

MS acquisition range: 50-1300 *m/z*
MS/MS acquisition range: 50-1300 *m/z*
Acquisition mode: Auto MS/MS & Fast polarity switching mode.
Ionization source: AJS ESI
Capillary Voltage: 3500V in both Positive and Negative mode.

Results and Discussion

LC-MS/MS system in the polarity switching mode identified system suitability standard, Dibutyl Phthalate. This standard has a mass of 278.1518 was showing *m/z* of 279.1607 in ESI positive mode and 277.1435 in ESI negative mode. The major fragment of m/z 279.1607 was *m/z* 149.0232 in ESI positive ionization mode.

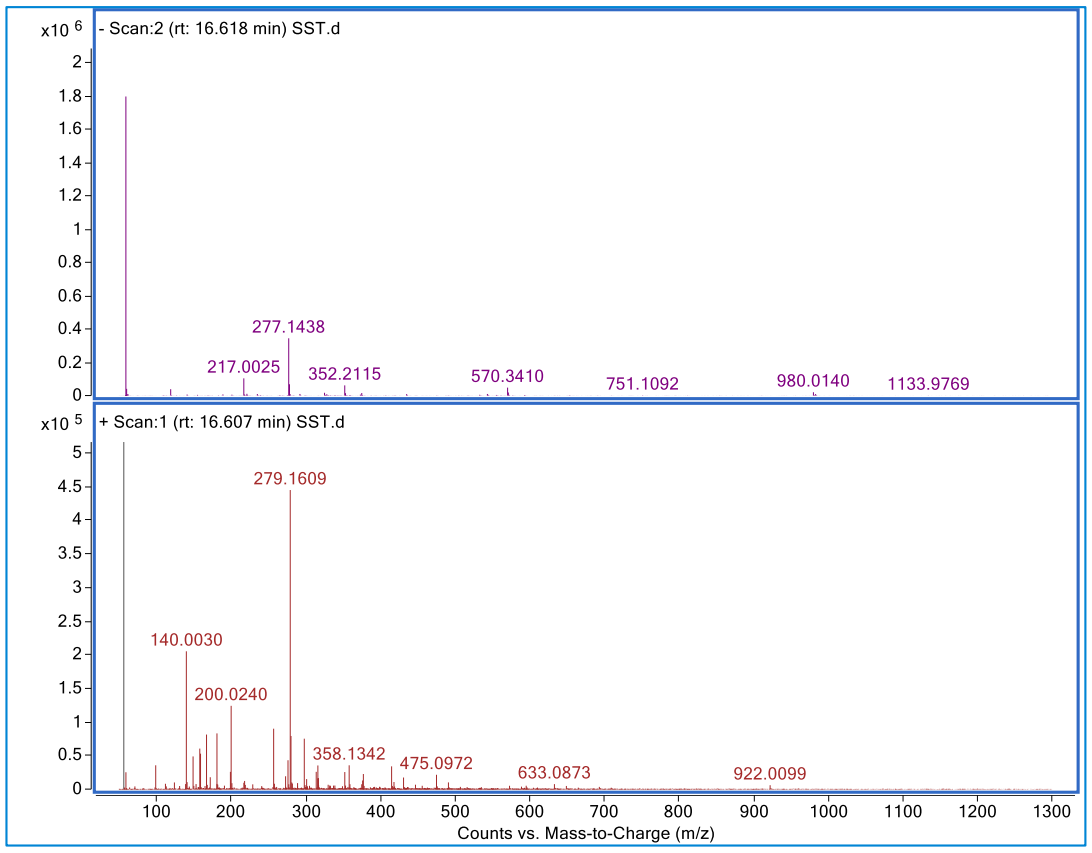

Figure 3. High resolution accurate mass spectra of Dibutyl Pthalate in polarity switching mode

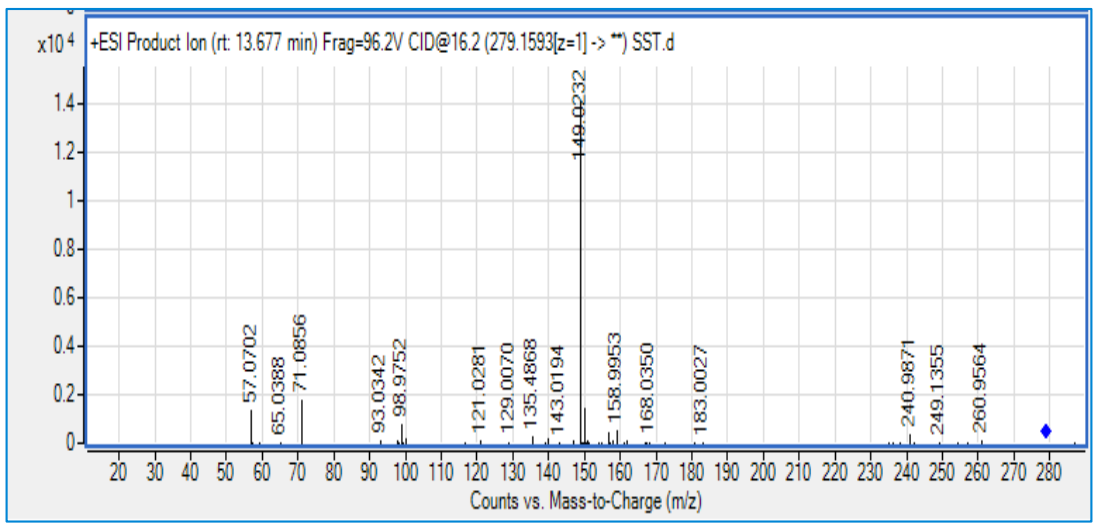

Figure 4. Fragmentation pattern of Dibutyl Pthalate generated in Auto MS/MS mode

Preliminary data showed the presence of a number of extractables in 3 components of the drug container. However, by comparing the total ion chromatogram of samples with respect to their controls, the major contribution of extractables was from the cap of the drug container.

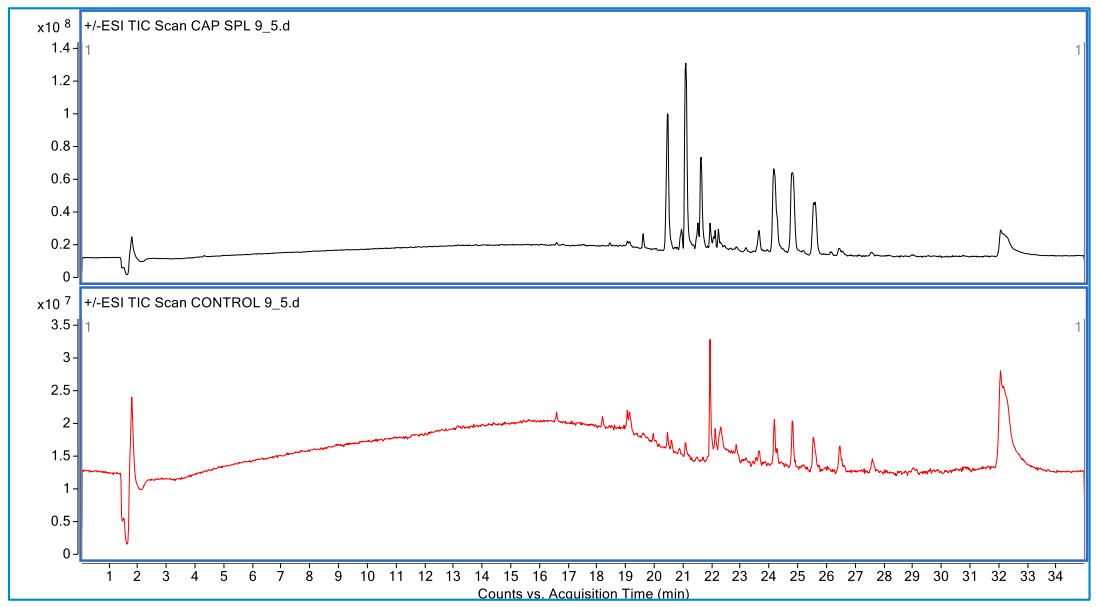

Figure 5. TIC of sample (Cap extracted with aqueous solution of pH: 9.5) vs. Control (aqueous solution of pH 9.5)

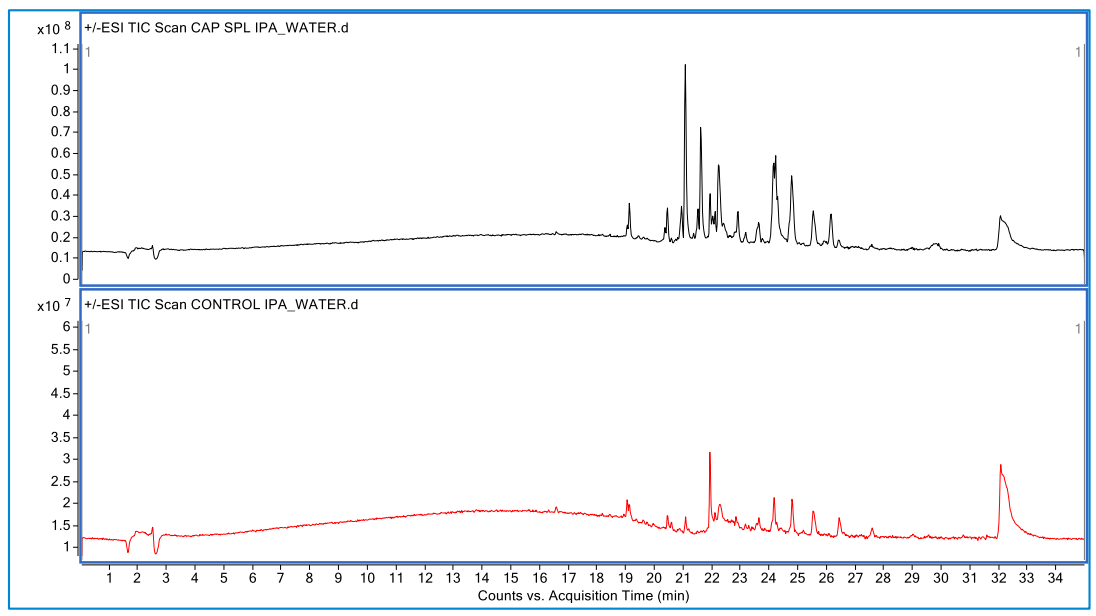

Figure 6. TIC of sample (Cap extracted with IPA: Water) vs. Control (IPA: Water)

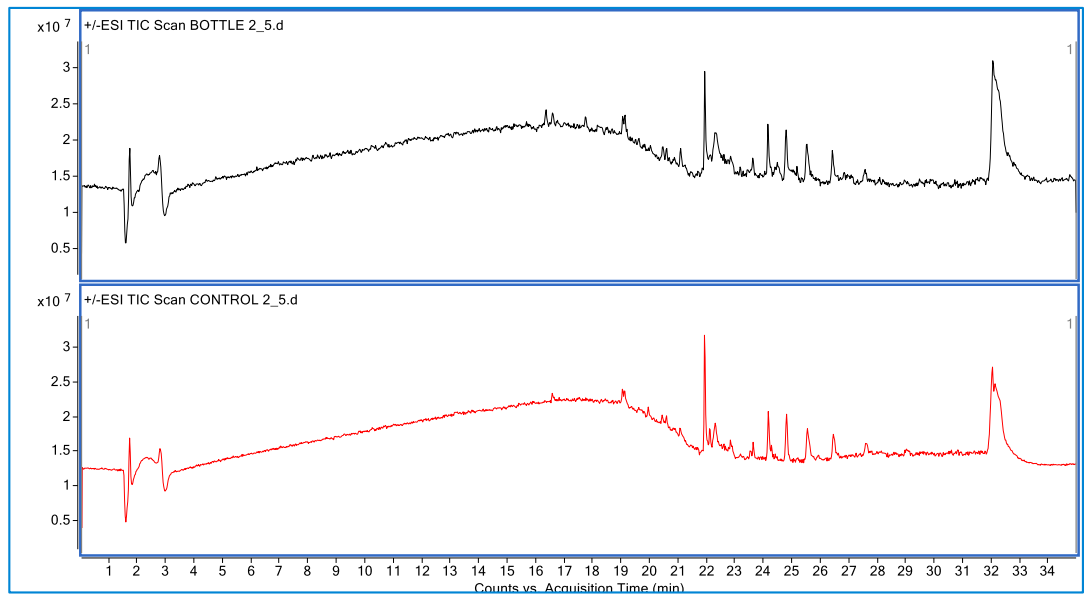

Figure 7. TIC of sample (Cap extracted with aqueous solution of pH: 2.5) vs. Control (aqueous solution of pH 2.5)

Results and Discussion

Identification of compounds at MS level

Screening by targeted approach confirmed the presence of extractables such as tetraethylene glycol, Irgacure 651, polypropylene glycol glycerol ether triacrylate, cyclohexylamine, 4-Isopropylthioxanthone and others.

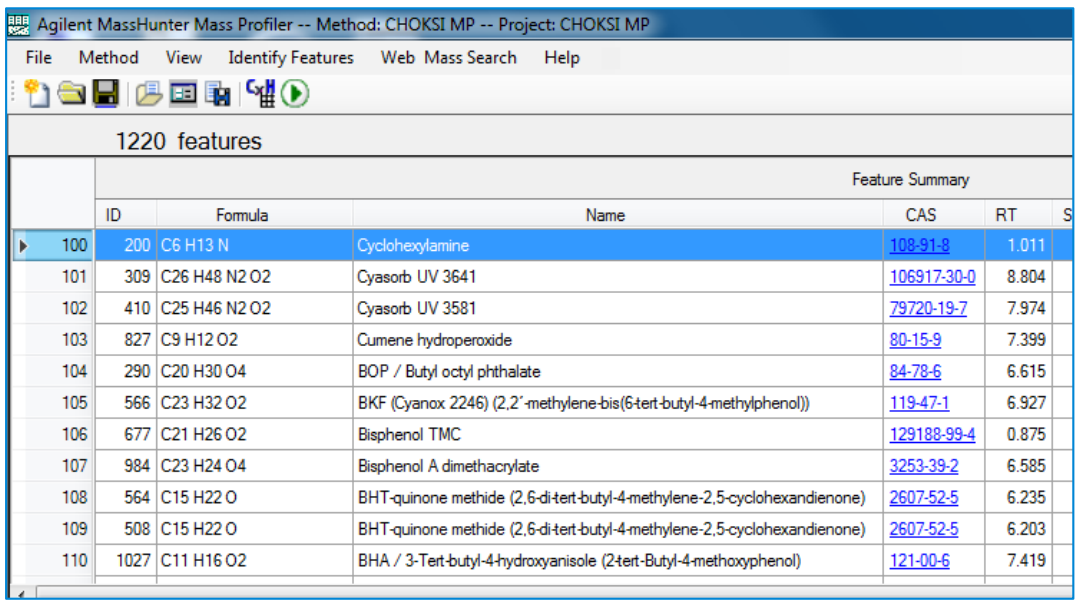

Figure 8. Screening result from Mass Profiler software and E&L database.

In order to check the agreement between the result obtained from MS-based screening and MS/MS-based identification, cyclohexylamine with a mass of 99.1047 was selected. The measured mass of 99.1040 was then searched in the NIST chemistry web book and one of the five results obtained was cyclohexylamine. Mass, 99.1047 of cyclohexylamine is obtained from the MS level screening by the accurate mass database is further taken for MS/MS analysis.

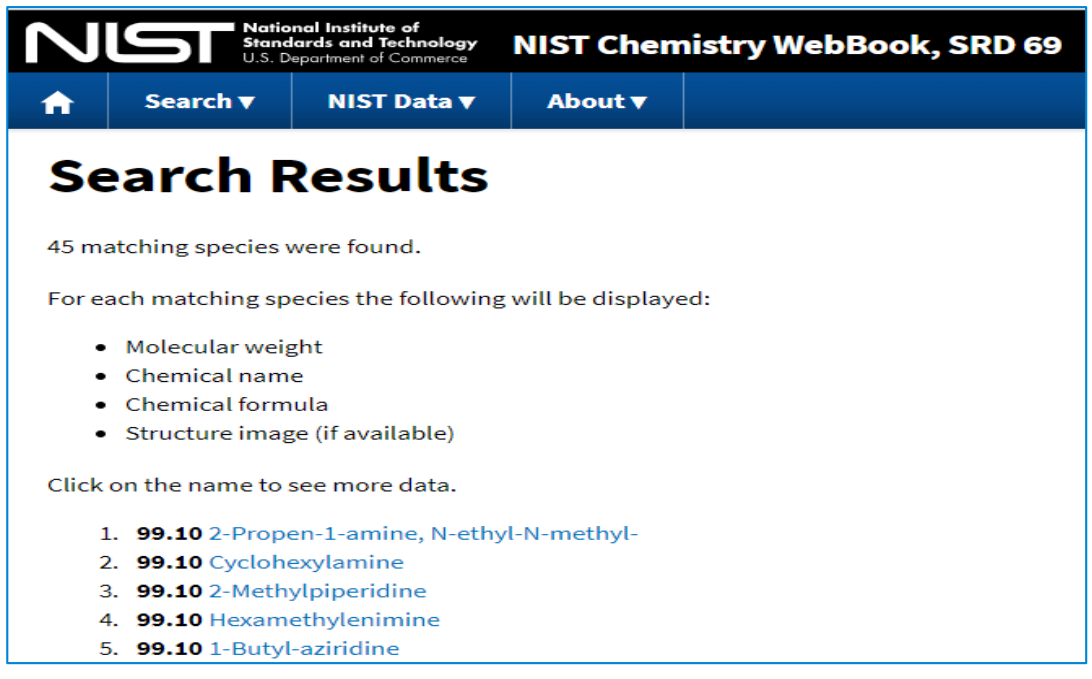

Figure 9. NIST webbook search result shows one of the possibility as Cyclohexyl amine

Confirmation of compounds at MS/MS level

Based on the fragmentation pattern obtained, molecular structure correlation software search results from the Chemspider database.

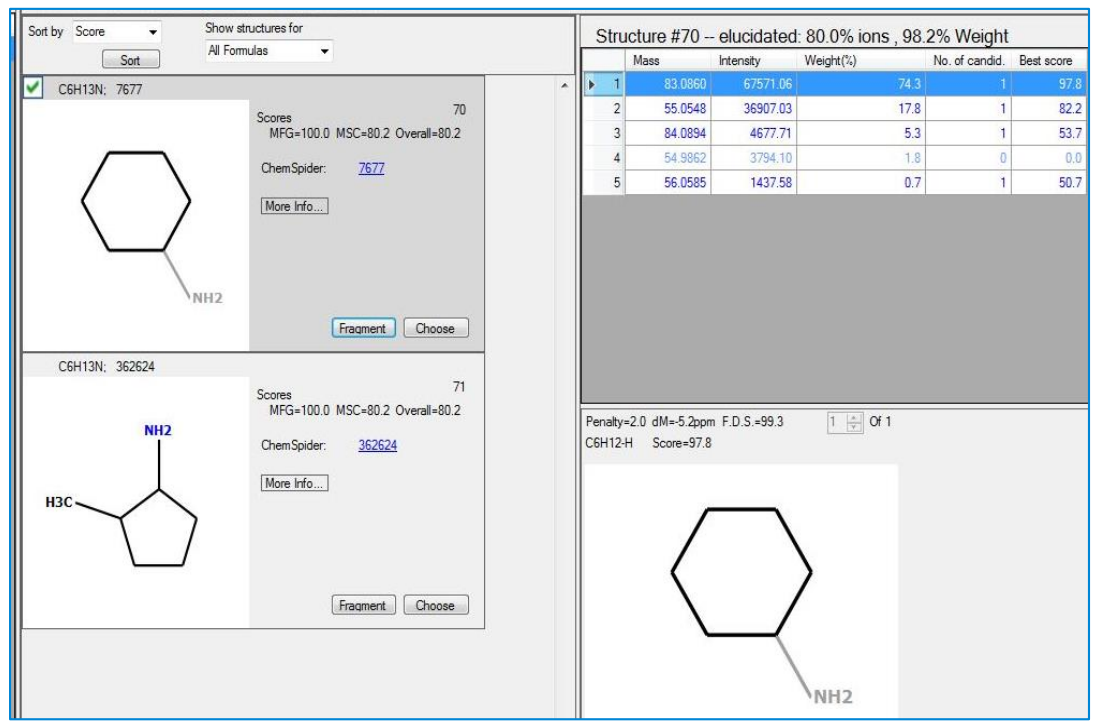

Figure 10. Probable structures given by Molecular structure correlator (MSC) software

Chemspider database result showed result id as 7677, which corresponds to cyclohexylamine. Confirmation of the same compound by both MS and MS/MS level increases the confidence in the results.

Conclusions

- Identification of extractables based on accurate mass increases confidence in the result.
- Confirmation of compounds at MS/MS level is demonstrated using MSC software
- Polarity switching mode increases the analysis throughput.

References

1. PDA Journal of Pharmaceutical Science and Technology, Vol. 67, No. 5, September-October 2013
2. Agilent Application note: 5991-6244EN

.

Published in USA, June 1, 2020

Agilent
Trusted Answers