A Generic UHPLC/UV/MS Method for Cleaning Verification of Highly Potent Drugs

Application Note

Pharmaceutical Quality Control (QC), Manufacturing of drug substances and drug products, GMP cleaning verification

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Abstract

Cleaning validation (CV) is a Good Manufacturing Practices (GMP) requirement in manufacturing of drug substances and drug products. Liquid Chromatography with UV detection is a common choice for cleaning verification studies for most drugs at sensitivity levels of approximately 20–50 ng/mL. For highly potent drugs, CV methods to reach sensitivity levels of low ng/mL can be implemented using UV detection with an extended pathlength flow cell or mass spectrometric detection. The sensitivity of UHPLC/MS method using an Agilent Jet Stream source on an Agilent 6150 Series Single Quadrupole LC/MS shows even lower limits of quantitation (LOQ) of approximately 0.5 ng/mL. In addition, higher injection volumes can further lower LOQs. This study shows a 2-minute generic cleaning verification method in a concentration range of 0.5 ng/mL to 1,000 ng/mL for five model drug compounds. This generic cleaning verification assay method can often be adapted to many different drugs with minimum efforts in method development, qualification, and transfer. This Application Note also shows the identification of unknown compounds employing a user-supplemented NIST Mass Spectral Search Program.

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Introduction

Cleaning verification (CV) must be performed to demonstrate the cleanliness of the production train (vessels and processing equipment) confirming that the active pharmaceutical ingredient (API) has been adequately removed to pre-established acceptance limits. The development of a high-sensitivity CV method is challenging for highly potent drugs (SafeBridge classifications 3B or 4[1]) or those possessing weak chromophores. For high potency drugs, acceptance criteria at low ng/mL detection limits are often required. The sensitivity of the traditional LC-UV method can be extended by using a long pathlength UV flow cell or mass spectrometric detection[2]. Additionally, unknown compounds are occasionally observed and should be identified in CV studies. Our objective was to demonstrate a fast, sensitive, generic UHPLC/UV/MS method to perform CV of highly potent drugs, and establish an easier mean to identify unknown compounds.

The Agilent 1290 Infinity LC System with UV Max-Light flow cell shows enhanced signals due to the extended pathlength. In earlier studies, a 60-mm Max-Light flow cell showed approximately 2–5x higher sensitivity than a standard 10-mm flow cell in cleaning validation studies using conventional HPLC columns[3,4]. We confirmed similar results in this study for the faster UHPLC method.

The Agilent 6150 Series Single Quadrupole Mass Spectrometer is also ideally suited for highly sensitive CV assays. The Agilent Jet Stream source (AJS) prevents further extra-column electrospray bandspreading in the source by collimating the spray with heated sheath gas, resulting in a sensitivity gain of up to 10 fold when compared to that of a standard electrospray ionization (ESI) source. The AJS source also handles a wider flow rate range of 20–2,000 µL/min. A high flow rate, used in CV method, can reduce analysis time without sacrificing peak capacities. The AJS source also has more effective ionization for different classes of compounds, compared to that of a regular ESI source. A single generic UHPLC/UV/MS CV method increases laboratory productivity of pilot-scale or manufacturing facilities,[5] which place strong demands on quick sample turnarounds.

In CV samples, different residual compounds, such as API from the current or previous batches, by-products, degradants, excipients, or cleaning agents can be found. An LC/UV/MS assay can also identify unknown peaks in the chromatogram with the aid of a user-created MS library. Agilent OpenLab ChemStation facilitates creating an add-on custom library to the NIST Mass Spectral Search Program.

This Application Note documents the development of a high-throughput UHPLC/UV/MS generic method using an Agilent Poroshell C18 column (3.0 × 30 mm, 2.7 µm) for five common drugs. This fast LC column packed with superficially porous particles has improved kinetic performance versus those with fully porous particles, and is operated at 1 mL/min for 2 minutes. Using this procedure, mixtures of the five model drugs at a concentration range of 0.5 ng/mL to 1,000 ng/mL were measured with the combined UV and MS method. Method performance, such as specificity, precision, accuracy, limits of detection (LOD), limits of quantitation (LOQ), and linearity, were determined and found to be acceptable for the intended use. The creation of a custom mass library and its searching capability were also demonstrated. This method can potentially be applied to a wide variety of drugs for CV with minimal efforts in method development, qualification, and transfer.

Experimental

A generic LC-UV/MS method was developed and demonstrated for five model drugs: sulfamethizol, sulfamethoxazole, propranolol, imipramine, and amitriptyline. These drugs were selected to illustrate method performance and system capability even though they are not considered highly potent. Linearity was shown at various concentrations using standard and extended pathlength UV flow cells and MS-SIM/SCAN mode. Studies using higher-volume injections were also performed to determine improvements in signal-to-noise ratio (S/N) for sulfamethoxazole. Table 1 shows the details of instrumental parameter of the generic method developed.

Reagents and Materials

All solvents and additives used were of LC/MS grade, purchased from Sigma-Aldrich (Saint Louis, MO, USA). Purified water was obtained from a Milli-Q water purification system from Millipore (Billerica, MA, USA).

Preparation of standards

The stock solution of sulfamethizol, sulfamethoxazole, propranolol, imipramine, and amitriptyline was prepared at 1,000 µg/mL prepared in 100 % methanol. The stock solution was diluted to 10 µg/mL working solution using 50:50 [methanol: mobile phase A (MPA)]. The linearity solutions at various concentrations were prepared by serial dilution. The concentrations in ng/mL were: 0.2, 0.3, 0.5, 1, 5, 10, 20, 30, 50, 100, 500, 1,000, 2,000, 5,000, and 10,000.

Table 1. The experimental parameters and instrumentation used in LC-UV/MS generic method.

Agilent LC/MS System	Parameter	Set value
Agilent 1290 Infinity Binary Pump (G4220A)	Mobile phase A	10 mM ammonium formate, 0.1 % acetic acid in water
	Mobile phase B	75/25 MeOH/ACN, 10 mM Ammonium formate, 0.1 % acetic acid
	Mobile phase gradient	0 minutes 10 % B, 1.5 minutes 90 % B, 1.6 minutes 10 % B, 2.0 minutes 10 % B
	Flow rate	1 mL/min
Agilent 1290 Infinity Autosampler (G4226A) maintained at 4 °C	Injection volume	5 µL (Needle wash for 10 s using ACN/Water 70/30)
Agilent 1290 Infinity Column Compartment (G1316C)	Temperature	40 °C
	Column	Agilent Poroshell 120 EC-C18, 3 × 30 mm, 2.7 µm
Agilent 1290 Infinity Diode Array Detector	Detection	280 ± 4 nm, 270 ± 4 nm, 260 ± 4 nm, 240 ± 4 nm Ref 400 ± 20 nm
Agilent 6150 Quadrupole LC/MS System (G6150BA) with AJS	Positive mode	SIM mode $(M+H)^+$: 271.1, 254.1, 260.2, 281.2, 278.2 Scan mode: 200–700
	Drying gas flow	10 L/min
	Nebulizer pressure	45 psig
	Drying gas temperature	300 °C
	Sheath gas temperature	200 °C
	Sheath gas flow	10 L/min
	Capillary cap	3,000 V
	Nozzle voltage	1,500 V
	Dwell time	47 msec
	Fragmentor/peak width	100/0.1 minutes
OpenLAB CDS ChemStation Edition Workstation (M8301AA)	Rev	C.01.05[36]
NIST Mass Spectral Search Program	Version	2.0 g

Results and Discussion

UHPLC/UV Detection

An Agilent 1290 Infinity Diode Array Detector using a high sensitivity, Max-Light 60-mm flow cell and a standard 10-mm flow cell was used alternatively in the generic UHPLC/UV method (Table1). The resulting chromatograms obtained in six replicate injections of a five-compound mixture using a 10-mm flow cell are overlaid (Figure 1). Linearity experiments were performed up to concentration 10,000 ng/mL using both flow cells. In the case of sulfamethizole, LOQs of 5 ng/mL and 20 ng/mL were found using a high sensitivity flow cell, and a standard flow cell respectively. As expected, a high sensitivity flow cell showed 3–4x lower limits of detection than that of a 10-mm flow cell. These results were comparable to an earlier study[2]. The concentration range, LOD/LOQ for both flow cell types are shown in Table 2. LOQs of 20–50 ng/mL are achievable using UV detection with a standard flow cell. The lineariy of the compounds in the experimental concentration levels shows correlation coefficient R^2 values of > 0.99 for the linearity ranged studies (Figure 2).

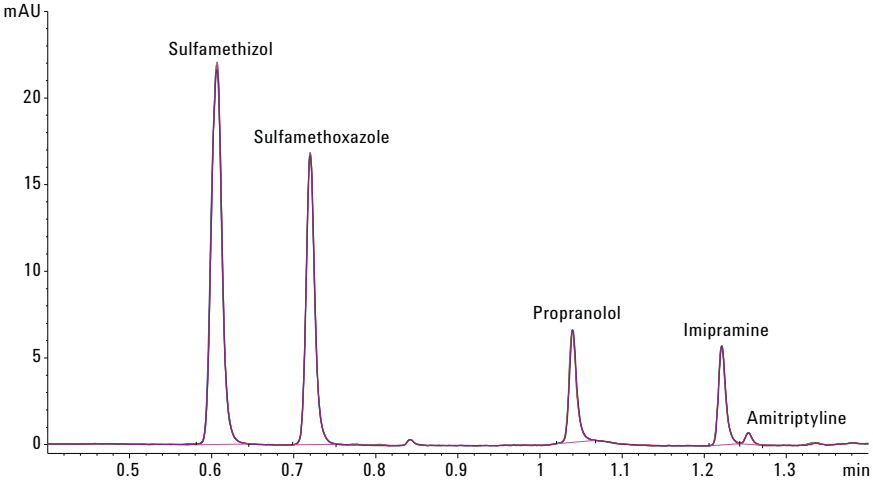

Figure 1. UHPLC/UV results. Reproducibility overlay of six chromatograms from replicate injections at 280 nm (1,000 ng/mL) using the standard 10-mm flow cell. The peak height for Amitriptyline is smaller since 280 nm is not optimal for that compound.

Table 2. UHPLC/UV method validation results using Max-Light High Sensitivity cell (60 mm) and standard cell (10 mm).

	LOD 10 mm	LOD 60 mm	LOQ 10 mm		LOQ 60 mm		Precision area 60 mm	Precision RT 60 mm	Correlation coefficient 10 mm	Correlation coefficient 60 mm
Compound	Conc (ng/mL)	Conc (ng/mL)	Conc (ng/mL)	S/N	Conc (ng/mL)	S/N	LOQ (RSD%)	LOQ (RSD%)	R^2	R^2
Sulfamethizole	10	1	20	27	5	23	5.8	0.15	1	1
Sulfamethoxazole	10	1	20	17	5	16	3.3	0.07	1	1
Propranolol	20	5	30	10	10	12	2.0	0.09	1	1
Imipramine	20	5	30	13	10	19	1.7	0.05	0.999	1
Amitriptyline	30	20	50	19	30	17	2.0	0.03	1	0.999

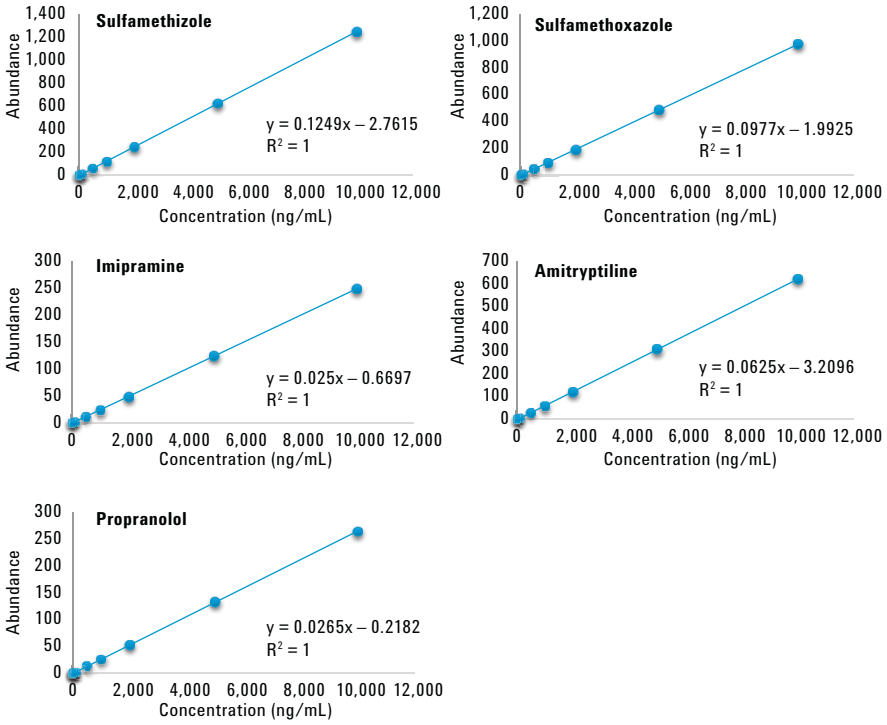

Figure 2. UHPLC/UV linearity runs using high sensitivity 60-mm flow cell.

Single quadrupole mass spectrometer (SQMS) detection

A 2-minute generic gradient method, as described in Table 1, was developed using an Agilent 6150 Single Quadrupole Mass Spectrometer with an AJS source. A chromatogram of the 30 ng/mL mixture is shown in Figure 3. The linearity experiments performed at 0.5 ng/mL to 100 ng/mL solution showed excellent linearity achievable at this range. The LOQ of 0.5 ng/mL obtained in this UHPLC/MS method is comparable or better to the sensitivity performance of a longer LC/MS method as described by Liu, *et al*. for highly potent drugs[6].

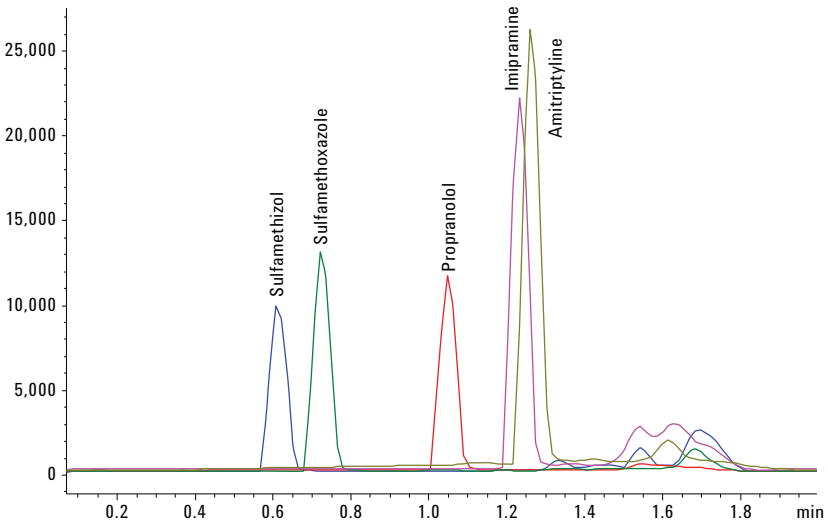

Figure 3. UHPLC/MS (SIM) results of 30 ng/mL mix solution.

The RSD of peak areas and retention times at LOQ are found to be less than 5 % and 0.1 % respectively (Table 3). The linearity of all compounds in the experimental concentration levels displayed coeffecients of linear correlation or R^2 values of > 0.98 (Figure 4). The accuracy values, back calculated from the linearity equation, were within 15 % using the weighting of $1/x^2$ (Table 4). The LOQ obtained from Single Ion Monitoring (SIM) method was also compared with MS scan data where both SIM and full scan were acquired within the same run. The LOQ of extraction ion chromatogram (EIC) of compounds from full scan data was 5 ng/mL for all compounds (data not shown) suggesting that SIM mode is ~10x more sensitive than that from the full scan mode.

High volume injections for LC/MS runs

The possibility to achieve even lower LOQs for a senstivie CV method was studied by using higher volume injections. This is useful when additional sensitivity is required. Higher-volume injections increased peak area and S/N, but may increase peak broadening. This may not be a major concern in CV determination since peaks associated with multiple APIs are rarely observed in actual CV sample solutions. The results for higher volume injections for three different concentrations of sulfamethoxazole using the generic method is shown in Table 5. The result showed that, with the increase in injection volumes on the 30-mm column from 5 µL to 12 µL, the peak area, peak width, and S/N increased by 57 %, 31 %, and 41 % respectively. The sulfamethoxazole study showed that another 2x increase in sensitivity can be achieved by using higher-volume injections of 10 µL. Higher volume injections beyond 12 µL would lead to peak splitting on the 3-mm column used in this study.

Table 3. The UHPLC/MS (SIM) method validation results.

Compound	[M+H]+	Linearity range (ng/mL)	LOD Conc (ng/mL)	LOQ Conc (ng/mL)	LOQ S/N	RSD area at LOQ (%) (n = 6)	RSD RT at LOQ (%) (n = 6)
Sulfamethizole	271.1	0.5–100	0.30	0.5	10	4.0	0.03
Sulfamethoxazole	254.1	0.5–100	0.20	0.5	11.3	3.5	0.02
Propranolol	260.2	0.5–100	0.20	0.5	24.3	2.3	0.02
Imipramine	281.2	0.5–100	0.20	0.5	13.3	3.1	0.01
Amitriptyline	278.2	0.5–100	0.20	0.5	10.0	4.9	0.01

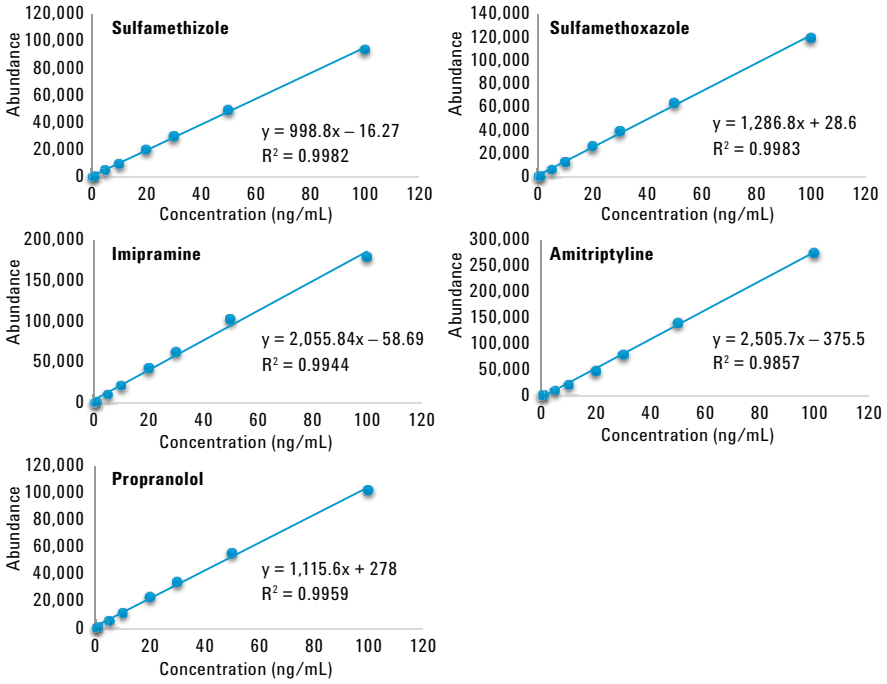

Figure 4. UHPLC/MS (SIM) method linearity of the drug compounds.

Table 4. UHPLC/MS (SIM) method accuracy.

	ng/mL							
Concentration	0.5	1	5	10	20	30	50	100
Sulfamethizole % mean accuracy (n = 6)	100.9	97.5	102.7	100.8	103.0	100.8	99.9	94.4
Sulfamethoxazole % mean accuracy (n = 6)	100.4	98.5	102.0	101.0	103.2	102.0	99.5	93.3
Propranolol % mean accuracy (n = 6)	101.0	97.3	100.8	103.0	105.0	102.9	99.5	90.4
Imipramine % mean accuracy (n = 6)	100.2	98.3	103.0	105.3	104.7	102.7	99.4	86.4
Amitriptyline % mean accuracy (n = 6)	106.5	90.3	86.1	89.5	97.6	106.6	112.9	110.5

User supplemented MS unit mass Library

When the UHPLC/MS method is used for CV, it is possible to detect unknown compounds in full scan mode. Finding an unknown peak may trigger further investigation. Software assisted identification of the compound from its mass spectrum would facilitate quick identification. A user-created unit mass MS spectral library was developed to augment the NIST Mass Spectral Search Program, an add-on software on OpenLab ChemStation Software. The spectra of five standard compounds were incorporated into the library. Sulfamethoxazole was injected, and a chromatogram was acquired in scan mode. The scan mode spectrum was searched within the user-created library. The results (Figure 5) show the correct identification of sulfamethoxazole. Therefore, unknown peaks can readily be identified from a NIST Mass Spectral Search Program supplemented with user-supplemented spectral information from reference standards.

Table 5. Peak area, peak width and S/N for higher volume injections of sulfamethoxazole solutions.

Concentration (ng/mL)	Injection volume (µL)	Peak area	Peak width (min)	S/N
10	5	4,012	0.09	1,787
	10	7,746	0.11	3,014
	12	9,160	0.13	3,031
20	5	7,484	0.09	3,508
	10	14,642	0.11	5,951
	12	17,581	0.13	5,985
30	5	10,838	0.09	5,152
	10	21,658	0.11	8,815
	12	25,924	0.13	8,885

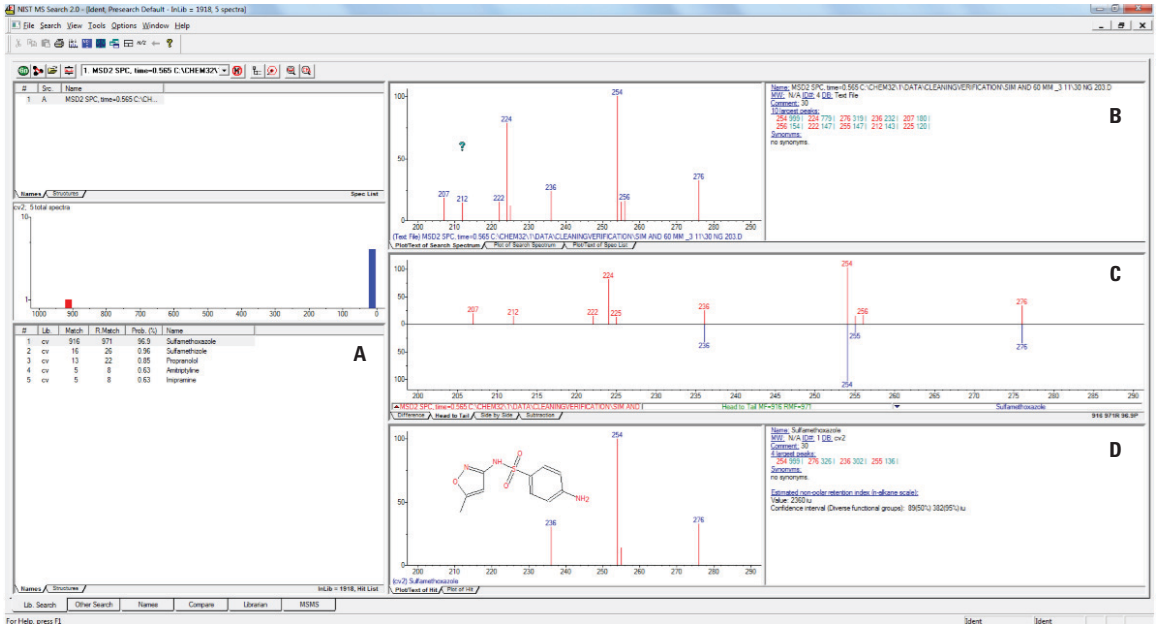

Figure 5. NIST mass spectral search program. A) Probability factor of 96.9 % that the identified compound is sulfamethoxazole. B) Spectra of unknown compound C) Compare view of the library compound and unknown compound D) Library spectra of the standard.

Conclusion

CV for high-potency drugs demands high-sensitivity and high-throughput methods. A 2-minute generic method for CV was developed and validated for five common drugs to illustrate the method performance for this application. LOQs of 20–50 ng/mL and 0.5 ng/mL were achievable using UV and MS (SIM) detection respectively. Further enhancements can be made using a Max-Light UV flow cell (2–4x) and larger volume injections (2x using 10 µL injection). An Agilent 1290 Infinity UHPLC and an Agilent 6150 Single Quadrupole Mass Spectrometer with an Agilent Jet Stream sources (AJS) operating under SIM or SCAN modes provided a versatile tool for CV studies for quantifying the rinsate solutions from manufacturing equipment. In addition, the identification of unknown compounds can readily be performed by searching from a user supplementary MS spectral library.

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Published in the USA, June 1, 2014
5991-4509EN

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