Access to **your** success
⊕ **SHIMADZU**

Analysis of Artificial Colorants by Ultra-Fast Liquid Chromatography-Mass Spectrometry

Technical Report vol.5

1. Introduction

There has always been a strong demand for high-throughput analysis. In liquid chromatography, the approach of using columns with finer packing material to decrease the theoretical plate height and increase mobile phase linear velocity has been effective in achieving higher throughput. However, careful consideration of such ramifications as the magnitude of the pressure loss and repeatability associated with the adoption of sub-2μm particle diameters as packing material is necessary.

In addition to shortening analysis time, enhanced separation and repeatability are also important factors. When column pressure is set to a value below 30 MPa, the best separation is achieved with a packing material particle diameter from 2.0 to 2.5μm. Based on this, we have developed the Ultra-Fast Liquid Chromatography (UFLC) system and a new series of 2.2μm-particle diameter ODS columns. Here we report on the evaluation of a UFLC-MS system used in the analysis of artificial colorants. The experiment is based on the permissible limits of the colorants and their approval for use as established in the Food Sanitation Law (Japan). The samples consisted of a mixture of twelve artificial colorants permitted under the law, and a mixture of thirty artificial colorants, twelve of which are those permitted under the law. For quantitative analysis, a photodiode array (PDA) detector was used, while an electrospray ionization / ion trap / time-of-flight mass spectrometer (ESI/QIT/TOFMS) was used for qualitative analysis.

2. Method

1) For evaluation of the LC-PDA system, the twelve synthetic colorant compounds permitted domestically were used (Fig.1).

2) Shimadzu's Prominence UFLC System, equipped with the Shimadzu Shim-pack XR-ODS (75 mm L x 3.0 mm I.D., 2.2μm) analytical column, was used. The mobile phase consisted of A: 50 mM ammonium acetate buffer solution (pH 4.7), and B: 100 mM ammonium acetate buffer solution (pH 4.7) / acetonitrile = 1/1, and gradient elution was conducted with a flow rate of 1.0 mL/min (10% B (0 min) - 50% B (0.5 to 0.6 min) - 100% B (2.6 to 3.0 min) - 10% B (3.01 to 6.5 min)). The injection volume was 4.0μL, and the detection wavelengths were 450, 520 and 620 nm.

3) The Shimadzu LCMS-IT-TOF was used for the LC-ESI/QIT/TOFMS measurement. The samples consisted of the twelve permitted colorant compounds, in addition to a mixture of thirty artificial colorants. The Shimadzu Shim-pack XR-ODS (75 mm L x 2.0 mm I.D., 2.2μm) was used as the analytical column, and the mobile phase consisted of A: 50 mM ammonium acetate buffer solution (pH 4.7), and B: 50 mM ammonium acetate buffer solution (pH 4.7) / acetonitrile = 1/1, and gradient elution was conducted with a flow rate of 0.5 mL/min (5% B (0 min) - 100% B (9 to 10 min) - 5% B (10.01 to 13 min)). The injection volume was 5μL. Accurate mass measurement (up to MS/MS) was conducted using the automatic polarity switching function, and formula composition was checked using the Formula Predictor software.
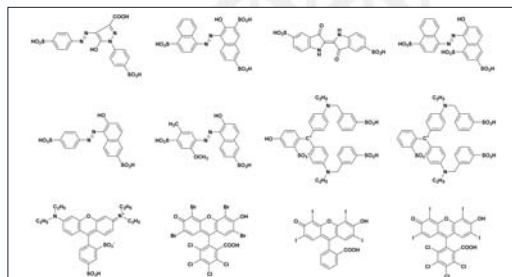

Fig. 1 Artificial Colorants

3. Results

1) In the UFLC-PDA measurement, analysis was completed in 3 minutes, shortened to approx. 1/6 the time required when using conventional LC.

2) The retention time repeatability (%RSD) and peak area repeatability (%RSD) were 0.11% and 0.15%, respectively, for all of the compounds, thus achieving shortened analysis time while maintaining accuracy (Table 1).
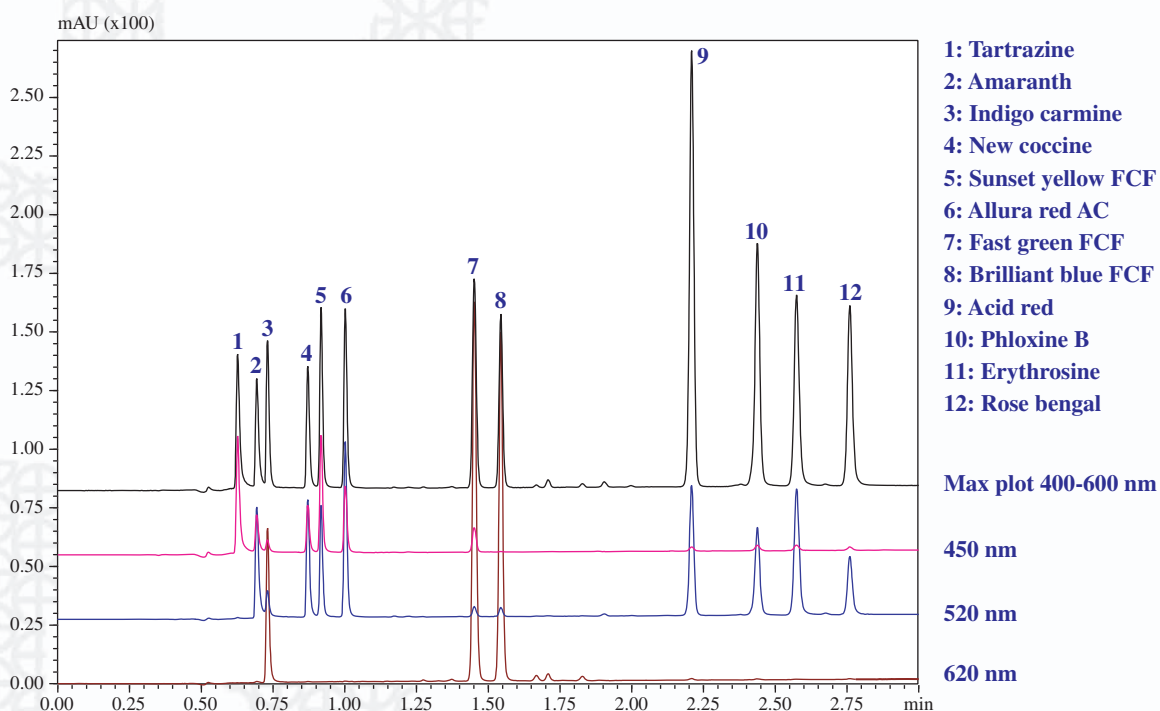

1: Tartrazine
2: Amaranth
3: Indigo carmine
4: New coccine
5: Sunset yellow FCF
6: Allura red AC
7: Fast green FCF
8: Brilliant blue FCF
9: Acid red
10: Phloxine B
11: Erythrosine
12: Rose bengal

Fig. 2 Representative PDA Chromatograms for Artificial Colorants by UFLC

Peak No.	Compound	Wavelength (nm)	Average of retention time(min) %RSD	Average of peak area %RSD
1	Tartrazine	450	0.626 0.101	43919 0.137
2	Amaranth	520	0.693 0.101	39058 0.142
3	Indigo carmine	620	0.730 0.065	47560 0.128
4	New coccine	520	0.87 0.078	37763 0.086
5	Sunset yellow FCF	450	0.916 0.074	36241 0.148
6	Allura red AC	520	1.001 0.057	56083 0.135
7	Fast green FCF	620	1.45 0.073	154295 0.100
8	Brilliant blue FCF	620	1.543 0.064	135981 0.107
9	Acid red	520	2.209 0.037	60262 0.100
10	Phloxine B	520	2.438 0.035	45077 0.125
11	Erythrosine	520	2.574 0.025	69000 0.140
12	Rose bengal	520	2.760 0.024	31176 0.109

Table 1 Repeatability of retention time, peak area (n=10, UFLC-PDA)

3) In the UFLC-ESI/QIT/TOFMS measurement, analysis was completed in 9 minutes (Figs. 3, 4).
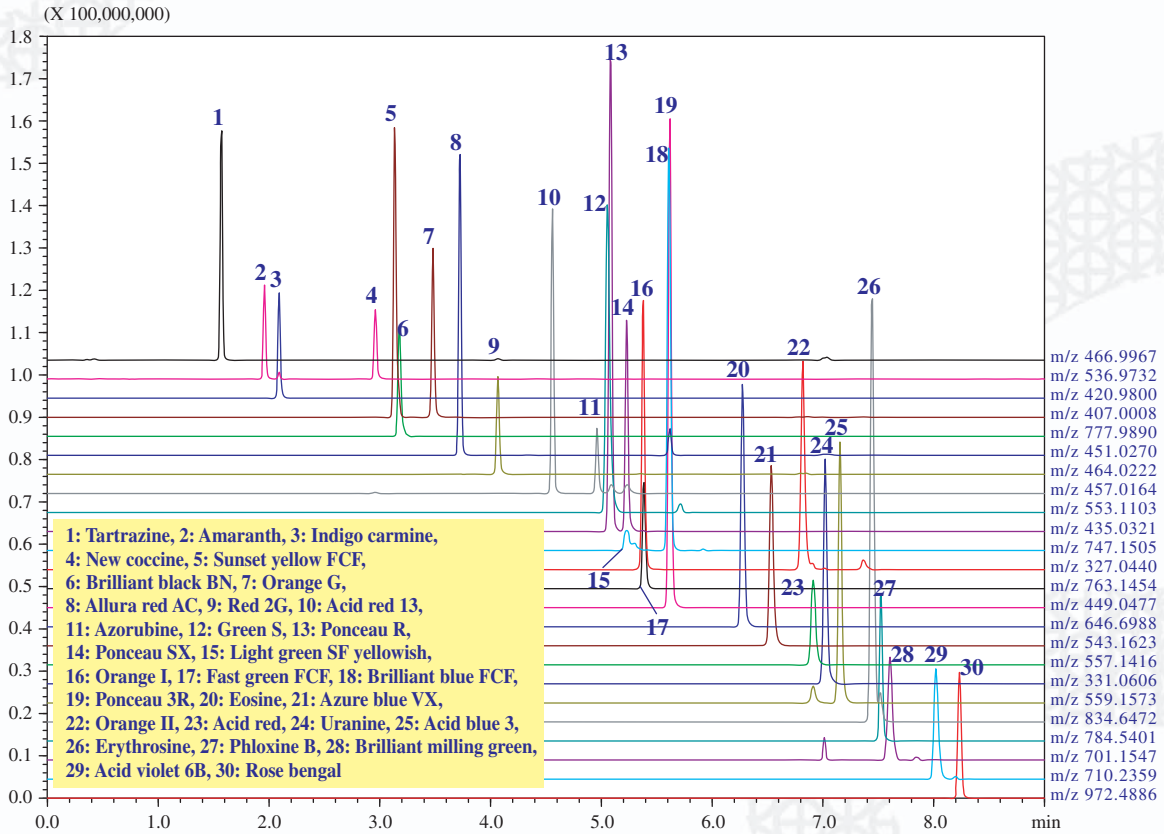

Fig. 3 Representative Mass Chromatograms for Artificial Colorants (Negative Ion Mode)
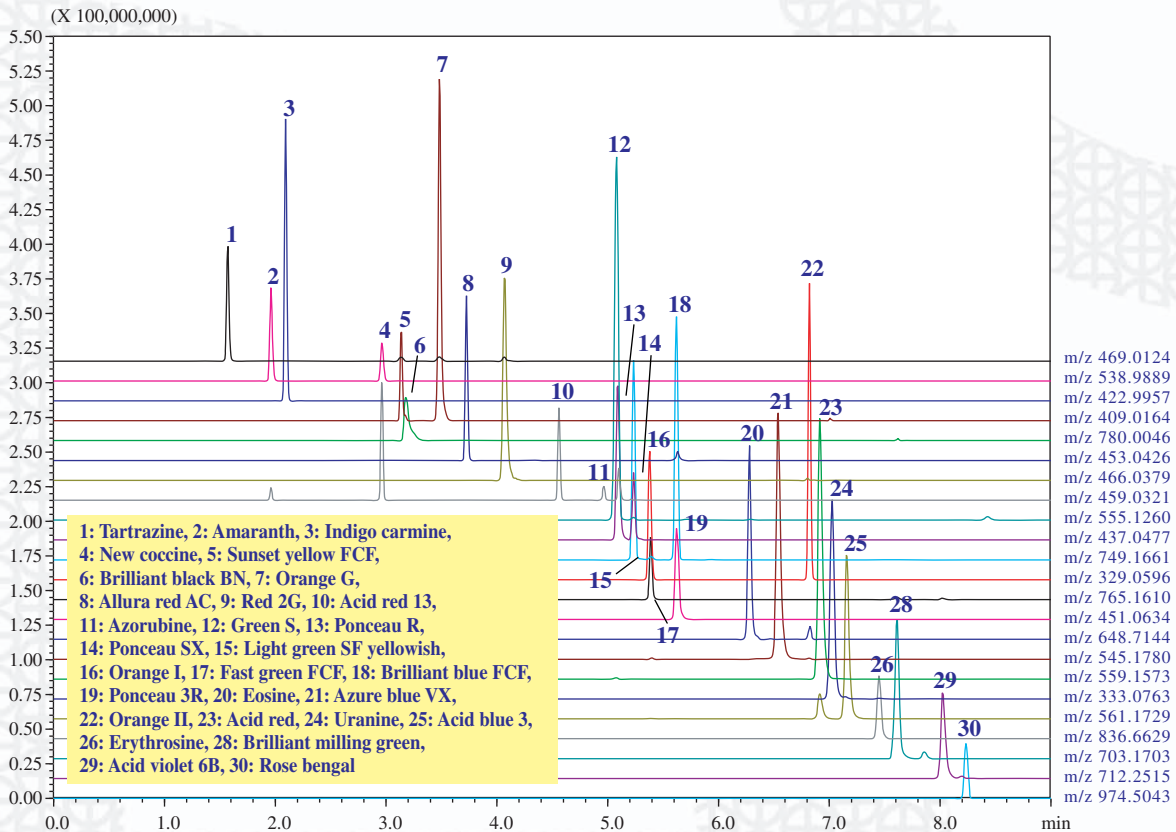

Fig. 4 Representative Mass Chromatograms for Artificial Colorants (Positive Ion Mode)

4) After conducting TOF calibration using an external standard, accurate mass measurements were taken using the automatic polarity switching mode. As indicated in the results (Tables 2, 3), the error observed was less than 10 ppm for all analytes in both positive and negative mode. Compounds with the same exact mass are categorized and displayed according to color.

Peak No.	RT (min)	Compound	Exact mass	m/z , theoretical	m/z , measured	Error (ppm)
1	1.566	Tartrazine	468.00457	466.99674	466.9984	3.55
2	1.955	Amaranth	537.98106	536.97323	536.9742	1.81
3	2.086	Indigo carmine	421.98786	420.98003	420.9776	-5.77
4	2.964	New coccine	537.98106	536.97323	536.9738	1.06
5	3.135	Sunset yellow FCF	408.00859	407.00076	407.0013	1.33
6	3.175	Brilliant black BN	778.99679	777.98896	777.9902	1.59
7	3.483	Orange G	408.00859	407.00076	407.0010	0.59
8	3.720	Allura red AC	452.03481	451.02698	451.0264	-1.29
9	4.063	Red 2G	465.03006	464.02223	464.0235	2.74
10	4.558	Acid red 13	458.02424	457.01641	457.0175	2.39
11	4.960	Azorubine	458.02424	457.01641	457.0182	3.92
12	5.020	Green S	554.11814	553.11031	553.1119	2.87
13	5.086	Ponceau R	436.03989	435.03206	435.0329	1.93
14	5.222	Ponceau SX	436.03989	435.03206	435.0322	0.32
15	5.234	Light green SF yellowish	748.15829	747.15046	747.1536	4.20
16	5.373	Orange I	328.05178	327.04395	327.0451	3.52
17	5.388	Fast green FCF	764.15321	763.14538	763.1489	4.61
18	5.605	Brilliant blue FCF	748.15829	747.15046	747.1513	1.12
19	5.615	Ponceau 3R	450.05554	449.04771	449.0482	1.09
*20	6.271	Eosine	643.71052	646.69880	646.6993	0.77
21	6.528	Azure blue VX	544.17018	543.16235	543.1639	2.85
22	6.816	Orange II	328.05178	327.04395	327.0446	1.99
23	6.917	Acid red	558.14944	557.14161	557.1439	4.11
24	7.023	Uranine	332.06847	331.06064	331.0587	-5.86
25	7.154	Acid blue 3	560.16509	559.15726	559.1564	-1.54
26	7.446	Erythrosine	835.65507	834.64724	834.6509	4.39
*27	7.527	Phloxine B	779.55463	784.54010	784.5460	7.52
28	7.608	Brilliant milling green	702.16250	701.15467	701.1558	1.61
29	8.021	Acid violet 6B	711.24368	710.23585	710.2393	4.86
*30	8.233	Rose bengal	971.49917	972.48860	972.4954	6.99

* Most abundant ion was chosen.
Table 2 Accurate Mass Measurement (Negative Ion Mode)

Peak No.	RT (min)	Compound	Exact mass	m/z , theoretical	m/z , measured	Error (ppm)
1	1.566	Tartrazine	468.00457	469.01240	469.0119	-1.07
2	1.955	Amaranth	537.98106	538.98889	538.9875	-2.58
3	2.086	Indigo carmine	421.98786	422.99569	422.9963	1.44
4	2.964	New coccine	537.98106	538.98889	538.9900	2.06
5	3.135	Sunset yellow FCF	408.00859	409.01642	409.0163	-0.29
6	3.175	Brilliant black BN	778.99679	780.00462	780.0064	2.28
7	3.483	Orange G	408.00859	409.01642	409.0164	-0.05
8	3.720	Allura red AC	452.03481	453.04264	453.0417	-2.07
9	4.063	Red 2G	465.03006	466.03789	466.0398	4.10
10	4.558	Acid red 13	458.02424	459.03207	459.0318	-0.59
11	4.960	Azorubine	458.02424	459.03207	459.0314	-1.46
12	5.020	Green S	554.11814	555.12597	555.1253	-1.21
13	5.086	Ponceau R	436.03989	437.04772	437.0497	4.53
14	5.222	Ponceau SX	436.03989	437.04772	437.0505	6.36
15	5.234	Light green SF yellowish	748.15829	749.16612	749.1695	4.51
16	5.373	Orange I	328.05178	329.05961	329.0614	5.44
17	5.388	Fast green FCF	764.15321	765.16104	765.1663	6.87
18	5.605	Brilliant blue FCF	748.15829	749.16612	749.1703	5.58
19	5.615	Ponceau 3R	450.05554	451.06337	451.0622	-2.59
*20	6.271	Eosine	643.71052	648.71440	648.7208	9.87
21	6.528	Azure blue VX	544.17018	545.17801	545.1780	-0.02
22	6.816	Orange II	328.05178	329.05961	329.0598	0.58
23	6.917	Acid red	558.14944	559.15727	559.1580	1.31
24	7.023	Uranine	332.06847	333.07630	333.0764	0.30
25	7.154	Acid blue 3	560.16509	561.17292	561.1725	-0.75
26	7.446	Erythrosine	835.65507	836.66290	836.6690	7.29
*27	7.527	Phloxine B	779.55463	786.55570	N.D.	N.D.
28	7.608	Brilliant milling green	702.16250	703.17033	703.1758	7.78
29	8.021	Acid violet 6B	711.24368	712.25151	712.2563	6.73
*30	8.233	Rose bengal	971.49917	974.50430	974.5084	4.21

* Most abundant ion was chosen. N. D. : Not Detected
Table 3 Accurate Mass Measurement (Positive Ion Mode)

5) Fig. 5 shows an example of formula prediction results. A high score was obtained by taking into account such information as mass accuracy, isotopic patterns, chemical rules (e.g. the nitrogen rule) and MS/MS measurement results (observed fragment ions). In the Fragment Info Results window shown below, more information regarding the observed product ions is shown, including possible formulae that could account for the observed ion and any complementary ions.
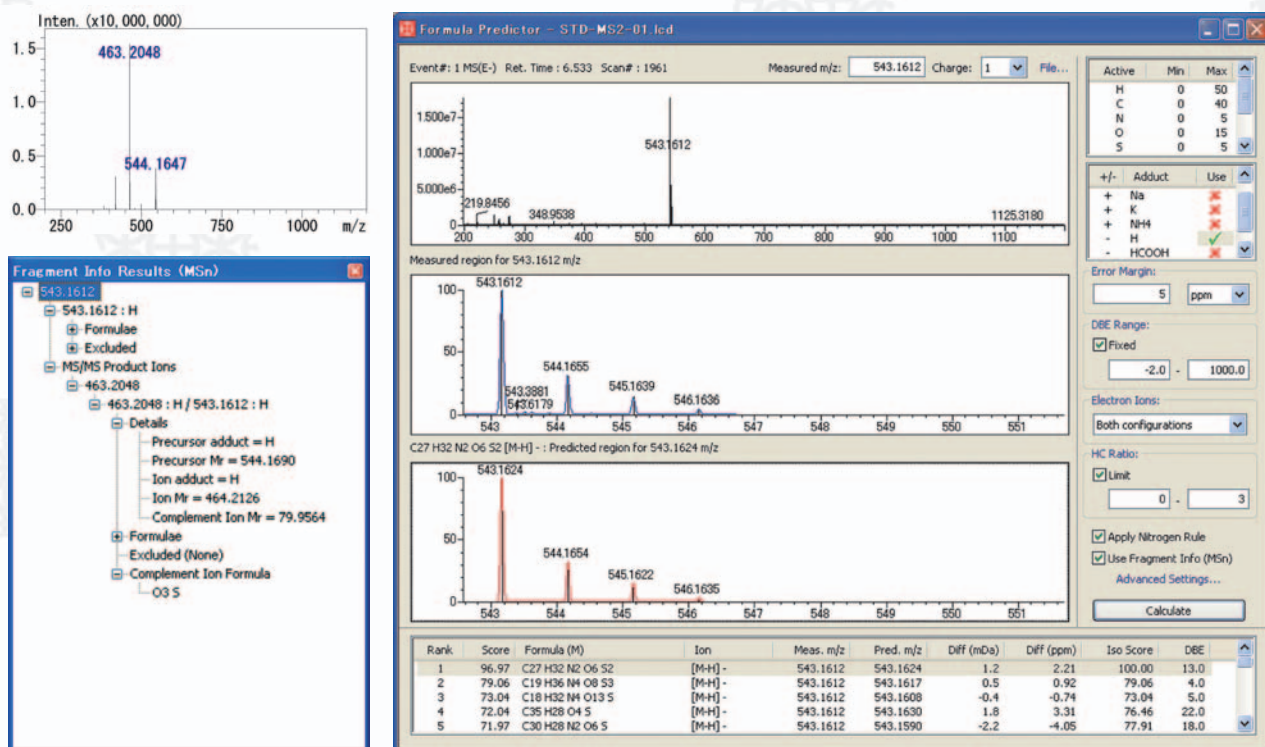

Fig. 5 Formula Predictor results for Azure blue VX, and the MS/MS spectrum observed after fragmentation of the parent ion, 543 m/z.

4. Conclusion

High-speed analysis of artificial colorants was conducted using the Prominence UFLC, achieving an analysis time that was shortened to 1/6 that obtainable using conventional LC. Excellent repeatability was also obtained. In addition, it was shown that compound identification could be conducted with greater certainty by combining accurate mass measurement and MS/MS measurement using a QIT/TOFMS, and that analysis time could be shortened by one-half by employing automatic polarity switching in the measurement.
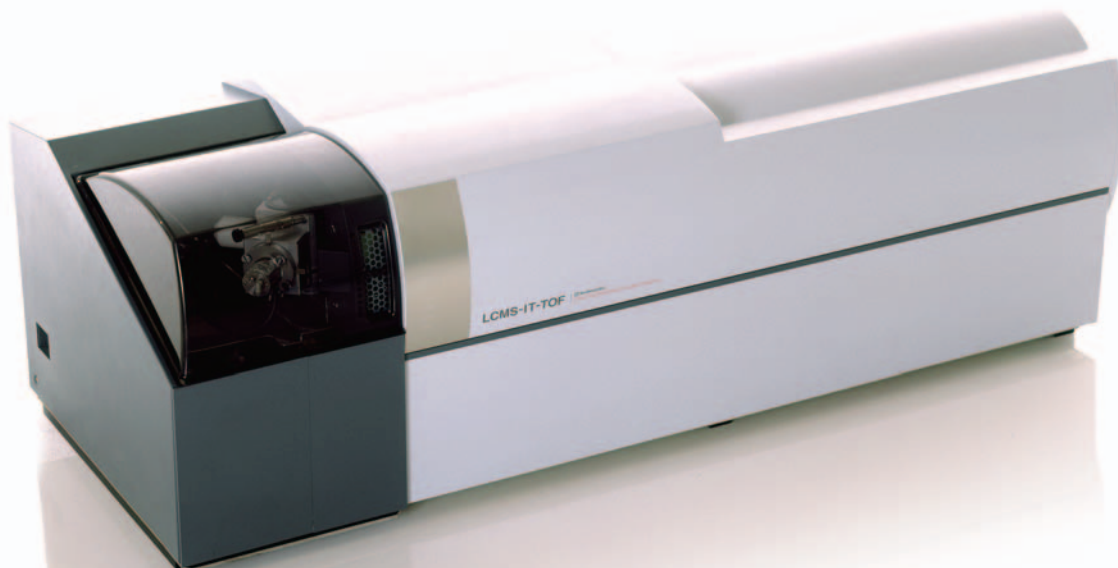
LCMS-IT-TOF LIQUID CHROMATOGRAPH MASS SPECTROMETER

Founded in 1875, Shimadzu Corporation, a leader in the development of advanced technologies, has a distinguished history of innovation built on the foundation of contributing to society through science and technology. We maintain a global network of sales, service, technical support and applications centers on six continents, and have established long-term relationships with a host of highly trained distributors located in over 100 countries. For information about Shimadzu, and to contact your local office, please visit our Web site at **www.shimadzu.com**

⊕ SHIMADZU