Analyzing Compounds of Environmental Interest Using an LC / Q-TOF Part 1: Dyes and Pigments

Application

Environmental

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Abstract

Dyes / pigments are produced worldwide and it is estimated that 10,000 tons are produced each year. About 10 percent of this is released into the environment in some form (such as the original compounds and degradants). Dyes and pigments have been identified as priority substances on the Chemical Management List by Environment Canada. There are many dyes / pigments in existence in the environment. An LC / Q-TOF is best suited to screen and identify many compounds in a single analysis.

Good mass accuracy (< 3 ppm) and MS / MS on an LC / Q-TOF provide powerful capability and high confidence to confirm ion identity and structure, useful for QA or unknown confirmation.

Introduction

In the past, environmental applications were carried out with GC, GC/MS, and other types of instruments. LC/MS did not have the sensitivity nor the robustness required for this field. However, in recent years, LC/MS technology has improved significantly and is now routinely used for environmental applications to monitor a list of hazardous compounds, for example, using LC/QQQ for screening and quantification of target compounds.

With the advent of new LC/MS techniques and improved performance, more compounds are being identified (new and emerging compounds). The compounds that are of interest for environmental analysis can be characterized by three categories:

1. Known knowns: the targets are known (for example, QQQ targeted analysis)
2. Known unknowns: it is known that compounds of interest are in a sample, but it is not clear what the compounds are (for example, metabolites, degradation products, or characteristic patterns/losses)
3. Unknown unknowns: it is not known what the compounds are and it is not know if they are present

A QQQ can be used to screen for category 1. However, a Q-TOF will be required for categories 2 and 3, where the compound must be identified. The routine accurate mass measurement allows a Q-TOF to find compounds via exact mass database search. The compounds found can be further confirmed by MS/MS on the Q-TOF.

Environment Canada is tasked with risk assessment and the evaluation of environmental impact of a variety of compounds [1, 2]. Dyes and pigments have been identified as priority substances on the Chemical Management List. Dyes/pigments are produced worldwide and it is estimated that 10,000 tons are produced each year. About 10 percent of this is released into the environment in some form (such as the original compounds and degradants). There are many dyes/pigments in existence in the environment; however, there is a limited number of standards available for analytical work. Standards can be produced, but the compound of interest must first be identified and characterized.

This study is to demonstrate the mass accuracy and Q-TOF's capability to generate useful formulas from accurate masses. In addition, MS/MS combined with accurate mass can be used to confirm ion identity and structure.

Experimental

Samples

The two classes of dyes/pigments studied for the application were azo dyes and anthracenediones.

The compounds that were evaluated were: Acid Blue 80, Acid Blue 129, Sudan Green 3, Toluidine Red, and Sudan III. These are the primary targets to be characterized; however, more sample categories will be added later for evaluation. The immediate concern is screening, which requires high mass accuracy for confirmation; the secondary concern is degradation products.

Instrument Parameters

All sample analyses were performed on an Agilent 1200 SL Rapid Resolution LC coupled to an Agilent 6520 Q-TOF.

All sample analyses were performed under Q-TOF autotune conditions. Mass accuracy, sensitivity, and resolution for all samples were measured without any changes to 6520 Q-TOF instrument parameters, except ion source conditions appropriate for the spray chamber type, LC flow, and sample thermal stability.

Mobile A	5 mM NH_4OAc, pH 4
Mobile B	MeOH
LC column	ZORBAX XDB 2.1 x 50 mm, C-18, 3.5 µm particle size
Flow rate	0.5 mL/min
MS	Scanned at 2 scans/sec, 50–1100 *m/z*
Positive ref. ions	*m/z* 121, 922
Negative ref. ions	*m/z* 113, 1034
AutoMS/MS	2 scans/sec MS and 2 scans/sec MS/MS
Q-TOF parameters	Set by autotune
Drying gas	13 L/min N_2 at 300 °C
Nebulizer pressure	50 psi
ESI (+)	3 KV
Fragmentor	140 V

Results and Discussion

One of the many ways to find compounds in a Q-TOF data file is by entering compound formulas to search. The formulas can be entered individually or as a group in a database in comma-separated value (CSV) format (see Figure 1). The database entry can include compound name, formula, and exact mass. Compound retention time is optional, but is very useful for getting results you can have confidence in. Figure 2 shows the spectra of the five matches using a mass tolerance of ± 5 ppm. The protonated ion is automatically labeled with the corresponding formula. Table 1 shows the mass accuracy (found by formula) of all five target compounds used in this study. All MH^+ and MNa^+ ions are within 3 ppm accuracy.

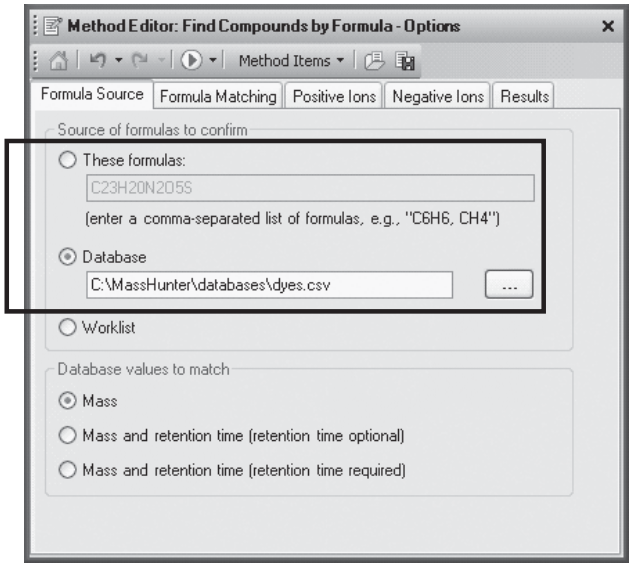
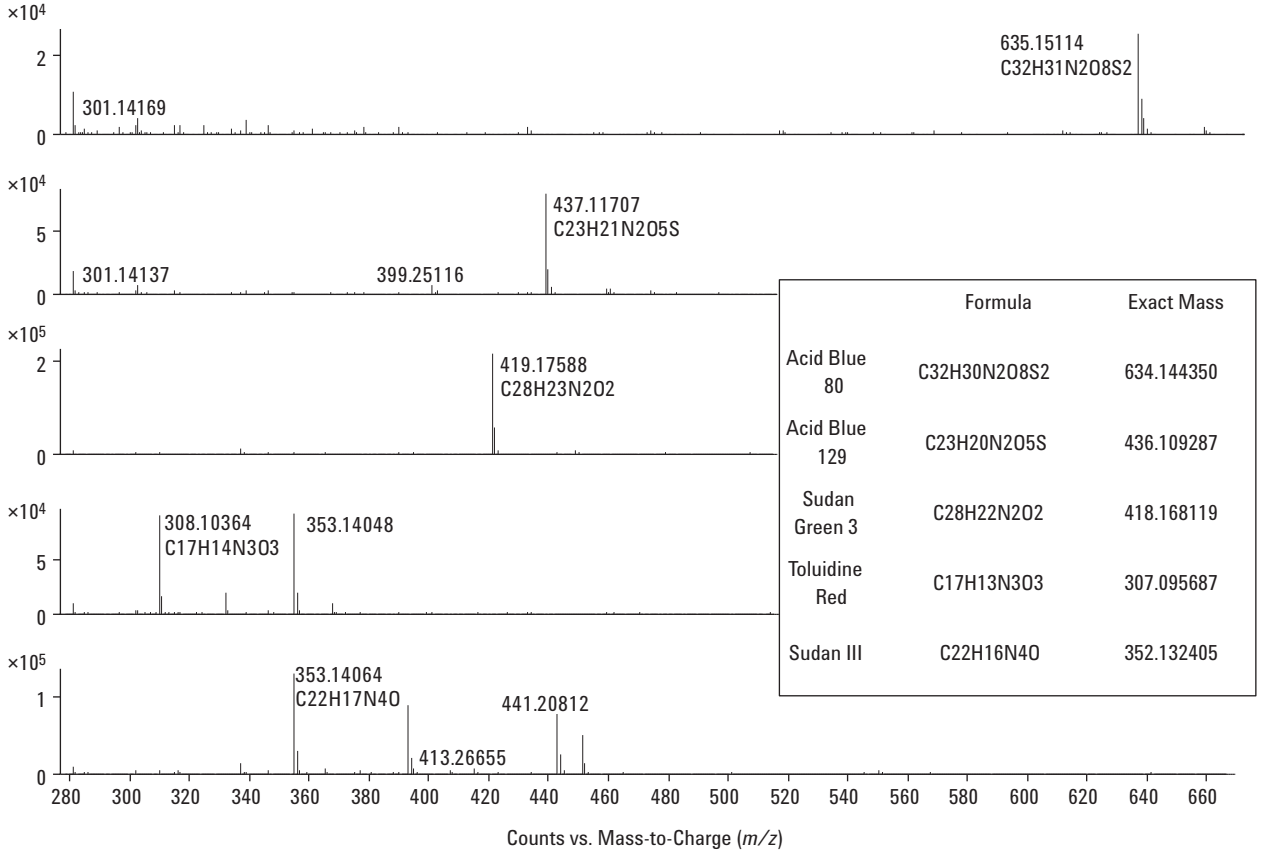


Figure 1. Find compounds in a Q-TOF data file by searching a manually entered formula or a formula database.



	Formula	Exact Mass
Acid Blue 80	C32H30N2O8S2	634.144350
Acid Blue 129	C23H20N2O5S	436.109287
Sudan Green 3	C28H22N2O2	418.168119
Toluidine Red	C17H13N3O3	307.095687
Sudan III	C22H16N4O	352.132405

Figure 2. The five dye compounds found by formula database search.

Table 1. The Mass Accuracy of the Five Compounds Found by Formula Match

	Formula	Exact Mass	Conc. (in MeOH)	MH^+	MNa^+
Acid Blue 80	$C_{32}H_{30}N_2O_8S_2$	634.144350	10 mg/L	0.33 ppm	0.40 ppm
Acid Blue 129	$C_{23}H_{20}N_2O_5S$	436.109287	10 mg/L	–1.13 ppm	–0.73 ppm
Sudan Green 3	$C_{28}H_{22}N_2O_2$	418.168119	10 mg/L	–1.65 ppm	–0.97 ppm
Toluidine Red	$C_{17}H_{13}N_3O_3$	307.095687	10 mg/L	–2.39 ppm	–1.69 ppm
Sudan III	$C_{22}H_{16}N_4O$	352.132405	10 mg/L	–2.89 ppm	–1.28 ppm

Another way to find compounds in a data file is by using Molecular Feature Extractor (MFE). This software program looks at ion characteristics and pulls out compounds from the total ion chromatogram (TIC). Figure 3 is an overlay of TIC and the 18 compounds (signal > 100 counts) found by MFE. To confirm the identity of each found compound, all compounds are searched against an exact mass database. Figure 4 shows the database search results where five out of 18 compounds had a match. Figure 5 is a screen capture of the Mass Hunter Software showing the five compounds identified from the exact mass database search. Two of the five compounds (both identified as Sudan III) at different retention times showed very similar spectra. By clicking the hot link built into the Mass Hunter software, a compound formula can be searched against several online databases for further confirmation. Figure 6 is the online ChemID database search results of Sudan III that showed two positional isomers that would likely give similar MS/MS information and four isomers that differ by more than position and would give dissimilar MS/MS information. Figure 7 shows the spectra from data-dependent MS/MS (auto MS/MS) that give automatic generation of MS/MS product ion formulas and loss formulas correlated with precursor ion formulas. The results show identical fragmentation of the two peaks, confirming that the two compounds were isomers of Sudan III.

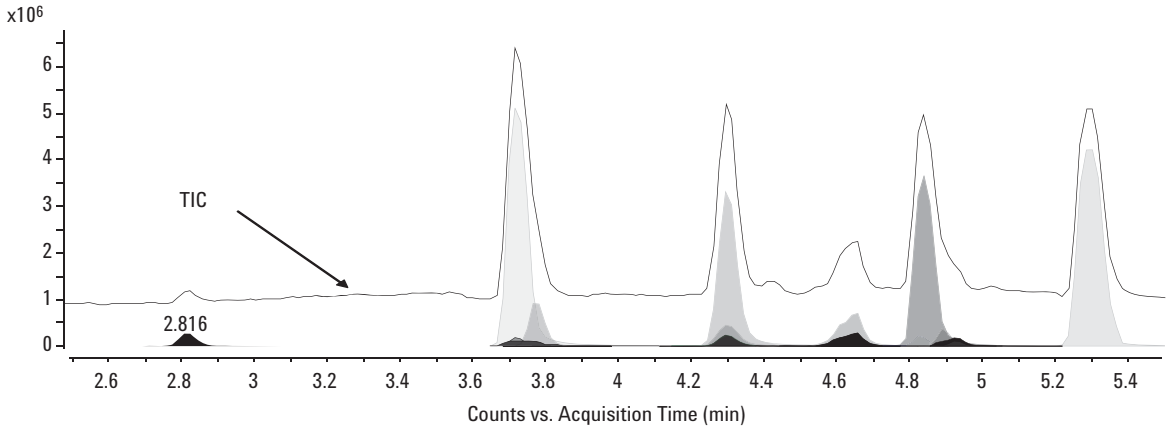


Figure 3. **Overlay of total ion chromatogram (TIC) and Molecular Feature Extractor (MFE) chromatograms of dye mixture. Eighteen compounds were found using the criterion of signal > 100 counts.**

Compound List

Name	RT	Mass	DB Formula	DB Diff (ppm)	Height
Cpd 1: Acid Blue 129	2.816	436.10981	C23H20N2O5S	-1.19	175661
Compound 2	3.72	307.25877			178040
Cpd 3: Toluidine Red	3.722	307.0967	C17H13N3O3	-3.27	3385650
Compound 4	3.733	329.07803			96154
Cpd 5: Sudan III	3.775	352.13362	C22H16N4O	-3.44	739956
Compound 6	4.299	232.1104			204562
Compound 7	4.3	334.2155			2067458
Compound 8	4.3	148.01592			191203
Compound 9	4.301	392.26843			364637
Compound 10	4.622	329.10603			141919
Compound 11	4.641	420.29968			210481
Compound 12	4.642	362.24654			471542
Compound 13	4.83	390.27811			156716
Cpd 14: Sudan III	4.84	352.13377	C22H16N4O	-3.86	2430832
Compound 15	4.893	440.2012			281429
Compound 16	4.921	390.27759			128443
Compound 17	4.928	157.05266			100077
Cpd 18: Sudan Green	5.296	418.16943	C28H22N2O2	-3.12	2842571

Figure 4. **Five compounds had hits from searching the Exact Mass Database.**

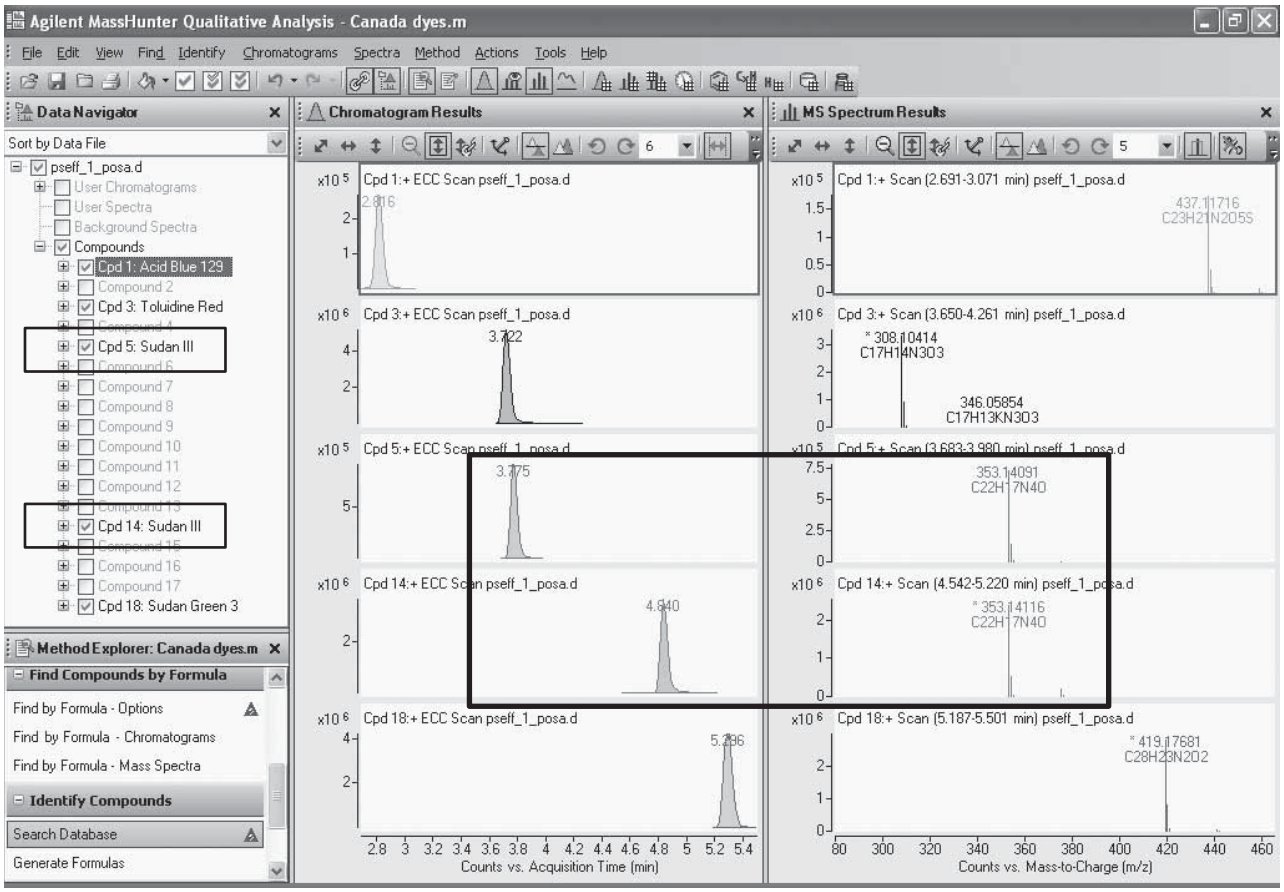


Figure 5. Mass Hunter software showing the five hits from the Exact Mass Database search.

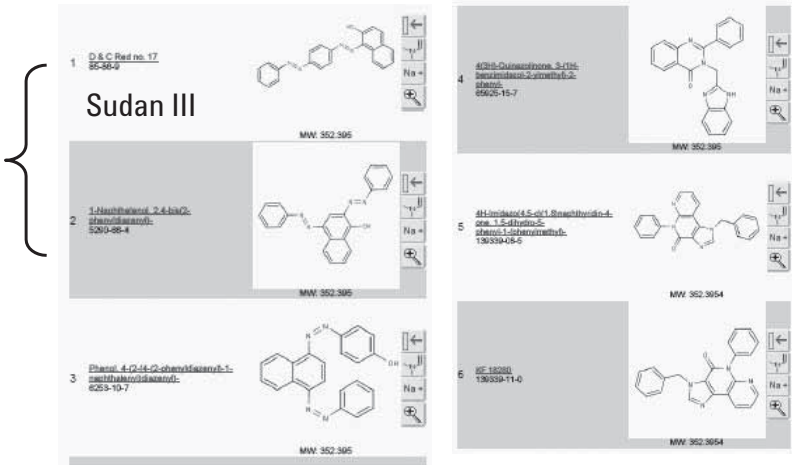


Figure 6. Online ChemID database search results from the Sudan III formula hot link.

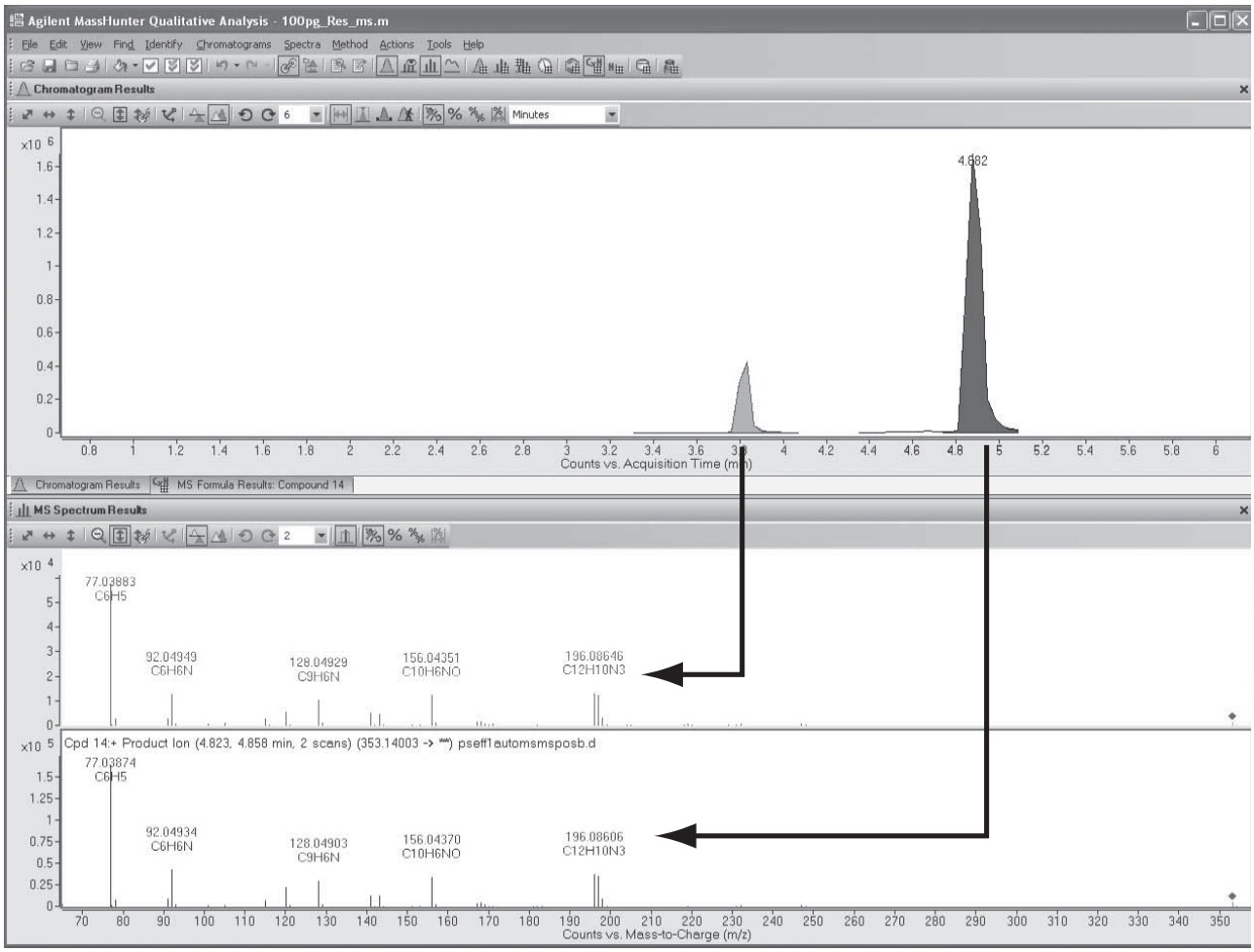


Figure 7. Examination of data-dependent MS/MS shows identical fragmentation from the two peaks, isomers of Sudan III.

Conclusions

- Compounds were found by either formula searching or by MFE and exact mass database searching, allowing a large number of compounds to be screened in a single analysis.
- Good mass accuracy (< 3 ppm) was achieved for five dye compounds, providing high confidence in results (formulas generated and compounds confirmed).
- MS/MS on Q-TOF provides powerful capability to confirm ion identity and structure, useful for QA or unknown confirmation.
- Hot link in Mass Hunter software allows quick formula searching against several online databases for compound identification and ease of use.

References

1. Jim Lau, Chin-Kai Meng, Jennifer Gushue, Robert J. Letcher, and Shaogang Chu, "Analyzing Compounds of Environmental Interest Using an LC/Q-TOF – Part 2: Fluorotelomer Unsaturated Acids," Agilent Technologies publication 5989-9132, August 2008.
2. Jim Lau, Chin-Kai Meng, Jennifer Gushue, Mark Hewitt, and Suzanne Batchelor, "Analyzing Compounds of Environmental Interest Using an LC/Q-TOF – Part 3: Imidacloprid and Manool," Agilent Technologies publication 5989-9129, August 2008.

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