A Total Solution for Explosives Analysis by Reversed-Phase HPLC with a Parallel HPLC System

G. Maio[1], F. Steiner[1], H. Franz[1], M. Tracy[2], X. Liu[2], F. Arnold[1]
[1]Dionex Softron GmbH, D-82110 Germering, Germany, giovanni.maio@softron.de,
[2]Dionex Corporation, Sunnyvale, CA, USA

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INTRODUCTION

The toxicity and persistence of explosive compounds has lead to increasing concern for the safety of the environment surrounding army firing ranges, munitions plants, and battlefields. The demand to analyze explosives and their degradation products has also increased for forensic analysis of terrorist activities. The EPA now regulates 14 explosives residues in Method 8330, recommending the use of a C18 reversed-phase column as the primary column for separating these compounds. It also requires confirmation of peak assignments using a secondary column with complementary selectivity. Therefore, the analysis involves running the same sample on two different columns, checking for correct peak assignments and reporting of quantification results. The requirements related to such a solution include:

- HPLC columns capable of separating all 14 compounds
- Hardware capabilities to combine the analysis on the primary and the confirmatory column
- Software which allows the automation of analysis on both columns
- Software which produces clear, concise, and understandable reports

This poster presents a total solution addressing all challenges listed above.

TOTAL SOLUTION APPROACH

General

In EPA regulated laboratories each sample for explosives analysis must be characterized by two independent methods. The use of two HPLC separations that take advantage of methods with orthogonal selectivity is a feasible approach for this. Two different HPLC separations can be achieved with the Dionex Acclaim® E1 und E2 explosives columns, each operated under distinct eluting conditions. An explosives sample passes the analysis procedure when the result of the quantitative analysis from column E1 can be confirmed by column E2.

The technical implementation and the analytical workflow with two independent LC methods can be accomplished in three different ways:

1. Use of two independent HPLC instruments to perform simultaneous analysis with column E1 and E2 for each sample
   - Achieves highest analysis throughput per day
   - Complete information including verification on each sample promptly available
   - Requires highest initial investment and running costs
2. Sequential performance of both methods on one instrument
   - Lowest initial investment and bench space allocation
   - Low analysis throughput per day
   - Every analysis in the E1 sequence lacks verification until the analysis on E2 can be performed
   - Significant manual labor requirement for replumbing of columns
3. Simultaneous application of E1 and E2 method in a parallel LC setup
   - Analysis throughput per day significantly increased
   - Complete information including verification on each sample promptly available
   - High degree of automation
   - Only slightly higher initial investment over a standard instrumental solution

Parallel LC Approach

The instrumental setup for parallel LC can be considered ideal for the analytical task. Although the system comprises only one extra module, the second detector, it performs almost like two independent HPLC systems. The operator installs both columns E1 and E2 in one column compartment, places the samples in one autosampler that is shared between the two systems, and then starts the two sequences simultaneously. After finishing both sequences, all information about the samples is readily available in the software. During analysis, each sample is simultaneously quantified with independent calibration.

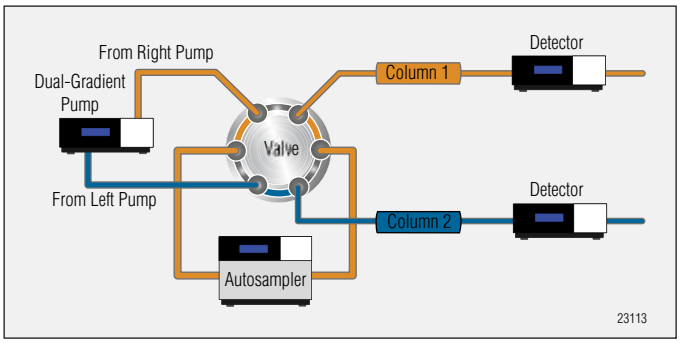

Figure 1. Flow scheme for UltiMate™ 3000 parallel LC mode. The two columns and the switching valve are located in one thermostatted column compartment.

The schematic setup is presented in Figure 1. The technical implementation comprises:

- Two independent flow paths that share one autosampler and one column compartment
- Chromeleon® software to provide intelligent management of the shared systems
- Two detectors to record the separation on both columns independently and simultaneously

The requirements for parallel LC with respect to method parameters are:

- The eluents must be fully miscible
- Both columns must be operated at the same temperature
- The initial eluent conditions on both columns should support fast flow path switching
- The runtimes for both methods should be similar

All these requirements can be fulfilled with the Acclaim E1 plus E2 HPLC solution for explosives analysis. Figure 2 compares the separation of the 14 explosives on the two columns and demonstrates their complementary selectivity. Peaks are labeled according to their elution order on column E1 and this labeling was maintained for column 2 (see Table 1 for peak identification). Elution conditions on both columns were optimized for application in a parallel LC setup. Both columns were operated isocratically at 31 °C and the compositions of the non-buffered eluents were similar to each other (43/57 v/v MeOH/H_2O on E1, 47/53 v/v MeOH/H_2O on E2). Switching the autosampler between the two flow paths transfers eluent of a slightly different composition to the channel where the complementary method is running. This can compromise the separation in the other channel and might adversely affect the quantification of target analytes due to small peak artifacts. Therefore, the valve switching times have to be selected carefully.

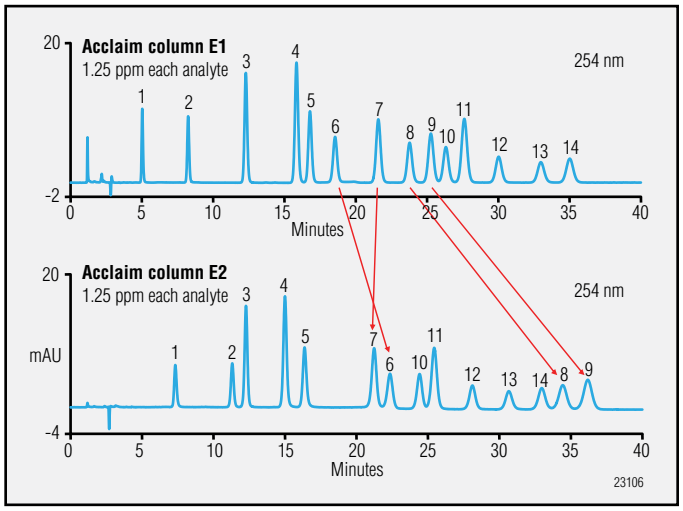

Figure 2. Separation of 14 explosives in parallel mode on Acclaim explosives eolumns E1 and E2 showing the complementary selectivity of E2 for confirmation of results from column E1. Both columns were operated at 1 mL/min and 31 °C. Eluent compositions were 43/57 v/v MeOH/H_2O on E1, 47/53 v/v MeOH/H_2O on E2. Injection volume was 50 µL. For peak identification, see Table 1.

Table 1. Peak Identification for Explosives Chromatograms Shown in Figure 2

Peak number	Compound
1	HMX
2	RDX
3	1,3,5-Trinitrobenzene
4	1,3-Dinitrobenzene
5	Nitrobenzene
6	Tetryl
7	2,4,6-Trinitrotoluene
8	4-Amino-2,6-Dinitrotoluene
9	2-Amino-4,6-Dinitrotoluene
10	2,6-Dinitrotoluene
11	2,4-Dinitrotoluene
12	2-Nitrotoluene
13	4-Nitrotoluene
14	3-Nitrotoluene

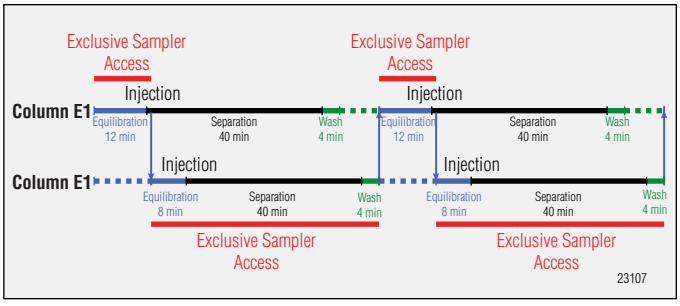

Figure 3. Schematic of the matched parallel analyses on E1 and E2.

The interplay of the 2 parallel HPLC methods is depicted in Figure 3. Shifting the autosampler to the alternative flow path was suppressed during the time window of exclusive sampler access (red bars). This precaution prevented the switching of an eluent plug while the other channel is performing a separation recorded by the detector. After switching the eluent plug to the other flow path (blue arrows), an optimized equilibration time period (12 or 8 min) was implemented (blue bars) to avoid any influence on the following separation. After completing the separation (black bars), a 4 min wash step at 80/20 v/v methanol/water was performed (green bars). Each sample was injected on both columns E1 and E2.

Two photodiode array detectors were used to record the UV spectra during the chromatographic separation. All explosive compounds exhibit characteristic UV spectra. Therefore peak identification was done by comparing the UV spectrum of the eluted peaks with reference UV spectra of the target analytes.

The peak retention time was used to highlight peaks eluting in the expected time window for each analyte but that could not be identified by their UV spectrum. This allowed for easy identification of potential false positive peaks. The reporting tools available in Chromeleon allow effective data reduction and data summary for an easy review of the results, despite the complexity of this application. Examples for peak characterization and reporting capabilities are shown in the "Results" section.

RESULTS

Retention Time and Area Precision

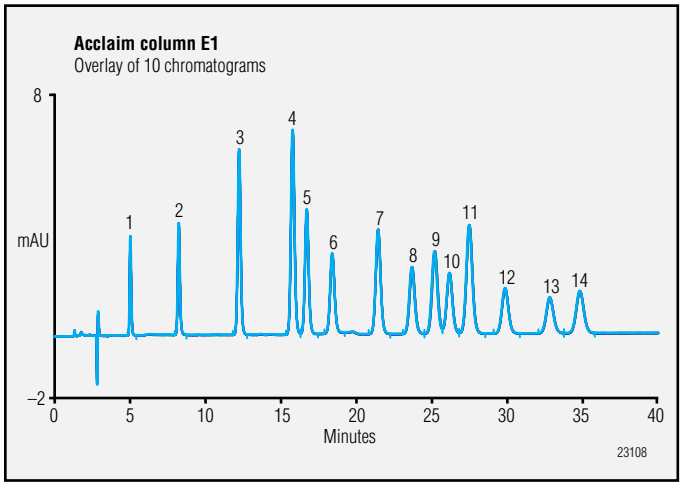

Figure 4. Overlay of 10 consecutive chromatograms on Acclaim explosives column E1 (50 µL injection of 14 explosives dissolved in water/methanol 60/40 (v/v), 500 ppb each). For peak identification, see Table 1.

Table 2. Retention Time and Peak Area Precision of Column E1

Compound	Column E1	
	RT %RSD	Area %RSD
HMX	0.000	0.37
RDX	0.047	0.21
1,3,5-Trinitrobenzene	0.028	0.30
1,3-Dinitrobenzene	0.025	0.14
Nitrobenzene	0.020	0.39
Tetryl	0.021	0.47
2,4,6-Trinitrotoluene	0.027	0.43
4-Amino-2,6-Dinitrotoluene	0.050	0.28
2-Amino-4,6-Dinitrotoluene	0.058	0.33
2,6-Dinitrotoluene	0.026	0.35
2,4-Dinitrotoluene	0.034	0.31
2-Nitrotoluene	0.023	0.57
4-Nitrotoluene	0.040	0.57
3-Nitrotoluene	0.040	0.52

Precision on both columns was comparable. As an example, Figure 4 shows an overlay of 10 consecutive separations on Acclaim Explosives column E1. Table 2 summarizes the retention time and peak area precision. The following results can be extracted:

- The retention time precision is <0.06% RSD for all compounds.
- The peak area precision is <0.6% RSD for all compounds, despite the low concentration levelof 500 ppb.
- The eluent inside the autosampler tubings can be switched between the two different methods with no impact on retention time precision.
- Small peak heights between 1 and 10 mAU and high injection volumes of easily outgasing sample solvents do not compromise peak area precision.

No Carryover

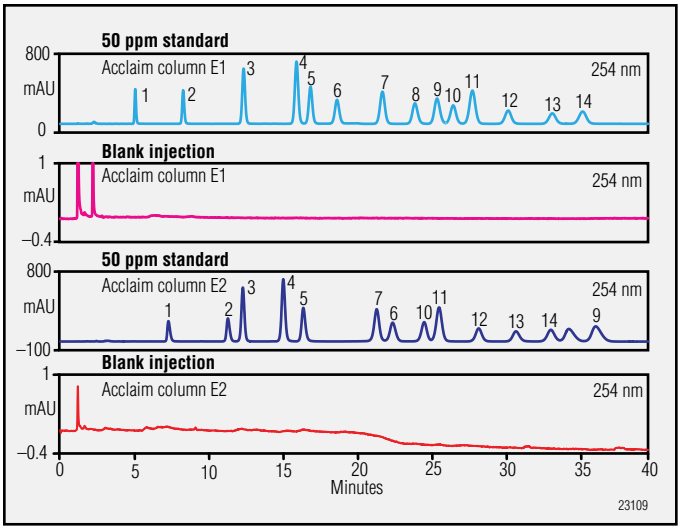

Figure 5. Fifty ppm standard injections followed by an eluent injection on Acclaim column E1 (top two chromatograms) and E2 (bottom two chromatograms) in parallel operation. For peak identification, see Table 1.

Figure 5 shows injections of a highly concentrated standard (50 ppm) followed by blank (eluent)injections on both columns. No carryover is observed when switching between the flow paths or from one injection to the other on the same column.

Calibration of Both Methods in Parallel LC Mode

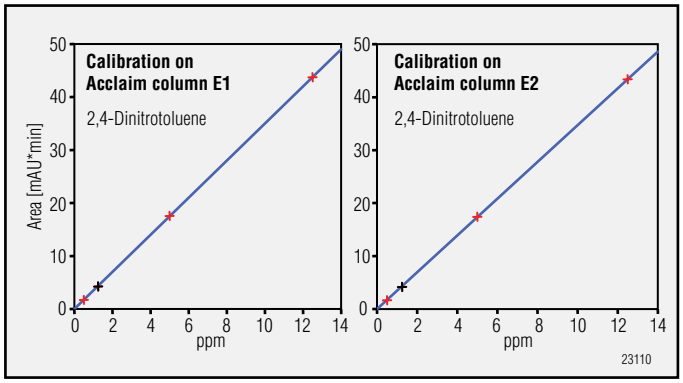

Figure 6. Calibration curves of 2,4-dinitrotoluene on Acclaim column E1 (left diagram) and E2 (right diagram), 50 µL injection of 0.5, 1.25, 5, and 12.5 ppm standards.

Table 3. Coefficient of Determination (r^2) and Relative Standard Deviations (RSD) for Calibrations on Both Columns E1 and E2

Compound	Acclaim Column E1		Acclaim Column E2	
	r^2	%RSD	r^2	%RSD
HMX	0.99994	0.91	0.99998	0.58
RDX	0.99998	0.61	0.99998	0.65
1,3,5-Trinitrobenzene	0.99996	0.70	0.99995	0.79
1,3-Dinitrobenzene	0.99998	0.53	0.99998	0.61
Nitrobenzene	0.99983	1.50	0.99984	1.45
Tetryl	0.99991	1.69	0.99997	1.15
2,4,6-Trinitrotoluene	0.99982	1.60	0.99993	0.97
4-Amino-2,6-Dinitrotoluene	0.99995	0.77	0.99998	0.69
2-Amino-4,6-Dinitrotoluene	0.99998	0.52	0.99998	0.71
2,6-Dinitrotoluene	0.99999	0.48	0.99999	0.61
2,4-Dinitrotoluene	0.99999	0.50	0.99998	0.66
2-Nitrotoluene	0.99973	1.99	0.99977	1.74
4-Nitrotoluene	0.99969	2.09	0.99966	2.12
3-Nitrotoluene	0.99956	2.54	0.99956	2.45

Table 3 lists the coefficients of determination and relative standard deviations of calibration curves for both columns. As an example, Figure 6 shows the calibration curves for 2,4-dinitrotoluene on column E1 (left) and column E2 (right) from 50 µL injections of 0.5, 1.25, 5, and 12.5 ppm concentrations. The results show excellent linearity for all listed compounds. The RSDs indicate a good method precision, especially considering the low concentration level of the target analytes.

Real-World Sample: Confirmation of Peak Assignments and Quantification

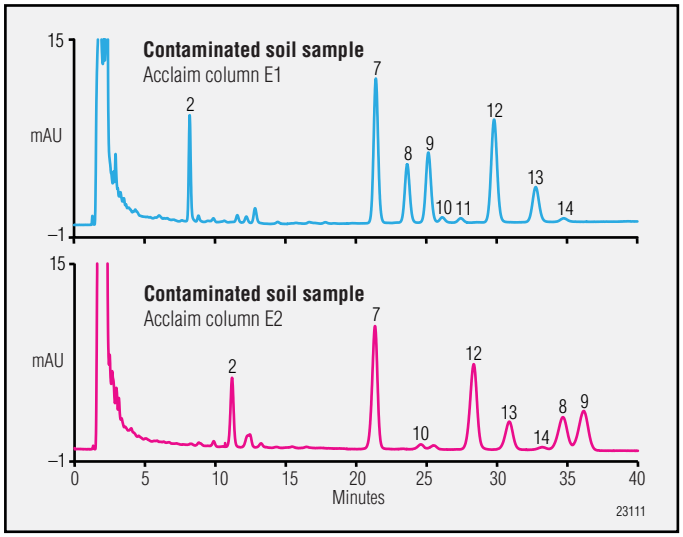

Figure 7. Contaminated soil sample (10 g soil extracted with 10 mL H_2O/MeOH 50/50 (v/v)), 50 µL injection volume on Column E1 (top chromatogram) and column E2 (bottom chromatogram). For peak identification, please see Table 1.

The real-world sample was successfully analyzed using the parallel LC approach described above. The same sample was automatically run on the primary column (Acclaim Explosives E1) and on the confirmatory column (Acclaim Explosives E2). The dual setup allowed virtually simultaneous processing of the two separate injections for each sample. Figure 7 compares the two chromatograms obtained. All identified target analytes are well resolved from each other and from residual matrix components.

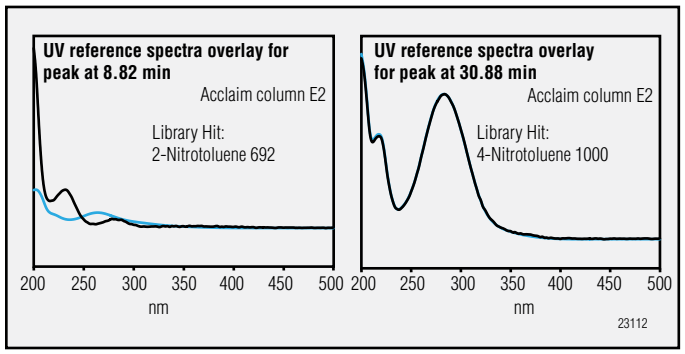

Figure 8. Overlays of soil sample peak spectrum and closest reference spectrum.

Figure 8 depicts two UV reference spectra match examples from the soil sample analysis on Acclaim column E2. The peak at 8.82 min (see left image in Figure 8) was not identified as one of the target explosives compounds, because the best found match factor of 692 is far below the acceptance threshold of 950. The visual comparison also shows that the two specta are different from one another. The peak at 30.88 min (see right image in Figure 8) was positively identified as 4-nitrotoluene (reference spectra match of 1000).

Table 4 shows an example of a Chromeleon report comparing the results of a contaminated soil sample analyzed on Acclaim column E1 and confirmed on Acclaim column E2. Results of column E1 are in blue, column E2 results are in red. For each column the following characterization criteria are displayed:

- **Reference spectra match**: The reference spectra match factor is an indicator of the similarity between the apex spectrum of an unknown analyte and a spectrum from a reference library. It varies from 0 (=no match) to 1000 (=perfect match). The target analytes are identified if the match is 950 or higher.
- **Peak purity**: The peak purity is an indicator for the spectral purity of a specific peak. As a quantitative measure, a UV spectral match factor is calculated. It refers to the correlation between the spectrum in the peak maximum (apex) and the spectra on the leading and trailing edges. It varies from 0 (=no match) to 1000 (=perfect match).

The presented total analysis solution yields the following information on explosives contaminated real-world samples:

- **Confirmed/Unconfirmed**: A compound is confirmed when identified by reference spectra match on both columns.
- **Peak Purity (pure ≥ 950)**: A peak is considered pure when the peak purity match on both columns is above 950.
- **Average Amount**: Calculates the average amount of a compound when quantified on both columns.
- **% Relative Amount Deviation**: Percent deviation of the amounts from the two analyses divided by the average amount.

Table 4. Chromeleon Report Table with Analyte Confirmation, Quantification/Peak Purity from Columns E1 and E2

Explosives Analysis Report

Sample: **Soil sample**

Column: **Acclaim Explosives Column E1** **Acclaim Explosives Column E2**

No.	Name	RT (min)	Peak Purity Match	Ref. Spectra Match	Amount ppm	RT (min)	Peak Purity Match	Ref. Spectra Match	Amount ppm	Confirmation	Peak Purity (Pure >= 950)	Average Amount	% Rel. Amount Deviation
2	RDX	8.217	1000	1000	1.15	11.183	1000	1000	1.15	Confirmed	Pure	1.15	0.00
7	2,4,6-Trinitrotoluene	21.433	1000	1000	1.57	21.333	1000	1000	1.47	Confirmed	Pure	1.52	6.37
8	4-Amino-2,6-Dinitrotoluene	23.658	1000	1000	0.99	34.692	999	1000	1.04	Confirmed	Pure	1.01	4.85
9	2-Amino-4,6-Dinitrotoluene	25.167	1000	1000	0.99	36.167	999	1000	1.00	Confirmed	Pure	0.99	1.17
10	2,6-Dinitrotoluene	26.158	960	994	0.10	24.583	881	988	0.11	Confirmed	Impure	0.11	7.44
11	2,4-Dinitrotoluene	27.458	906	956	0.05	n.a.	n.a.	n.a.	n.a.	Unconfirmed	Impure		
12	2-Nitrotoluene	29.833	1000	1000	3.01	26.350	1000	1000	2.78	Confirmed	Pure	2.89	8.20
13	4-Nitrotoluene	32.792	999	1000	1.30	30.875	999	1000	1.20	Confirmed	Pure	1.25	8.21
14	3-Nitrotoluene	34.792	922	994	0.11	33.200	919	980	0.10	Confirmed	Impure	0.11	5.76
1	HMX	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Not Present			
3	1,3,5-Trinitrobenzene	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Not Present			
4	1,3-Dinitrobenzene	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Not Present			
5	Nitrobenzene	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Not Present			
6	Tetryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Not Present			

From the results presented in Table 4, the following conclusions can be drawn for the analyzed contaminated real-world sample:

- Eight target analytes were positively identified on the primary column and confirmed on the second column.
- Six of these identified peaks showed peak purity above the defined threshold of 950 and are thus considered pure.
- 3-Nitrotoluene was identified on column E1 and E2. The peak purity match, however, was below 950 in both cases. Thus the peak was considered impure but was also quantified.
- 2,4-Dinitrotoluene was only identified on column E1 (reference spectra match) but could not be confirmed on the second column. Thus the peak is listed as "unconfirmed".
- The reported relative amount deviation is below 8.5% for all positively identified target analytes.

CONCLUSIONS

- A fully automated total solution for the analysis of explosives residues in accordance with EPA method 8330 is available.
- Operating the Acclaim Explosives Columns E1 and E2 in parallel mode resulted in a chromatographic performance equivalent to the sequential separate approach (i.e., both methods on one system).
- Unlike the sequential separate approach, the primary and confirmatory runs are processed under identical environmental conditions.
- The parallel LC approach provides significant productivity gains through the automated analysis of both the primary and confirmatory runs, and through the comprehensive reporting of both sets of results.
- The use of UV spectrum to identify the analytes prevents reporting of false positives.
- The trace level analysis of a real-world sample demonstrated good correlation between the two orthogonal methods.