Chromatography Technical Note No AS125

Large Volume Injection of selected explosives using the GERSTEL CIS 4 PTV Inlet and Agilent 5975C GC-MS.

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Introduction

A large volume injection (LVI) method has been developed for the analysis of five explosive compounds. Analytes were diluted in dichloromethane (DCM) & isopropyl alcohol (IPA), resulting in two slightly different analytical methods.

During the development of this method particular attention was paid on Cooled Inlet System (CIS) liner choice. The use of analyte protectants (keeper) was also investigated, when using DCM as the injection solvent due to the boiling point differential between DCM and the lowest boiling analyte.

After a lengthy development period, two 100 µl LVI methods have been developed for the injection of explosive extracts in DCM and IPA.

Instrumentation

Agilent GC-MSD 5975C inert MSD with Triple-Axis Detector EI source (Agilent GC 7890A).
GERSTEL MPS 2 XL-*xt*
10 or 100 µl GC Syringe
GERSTEL CIS 4 PTV fitted with septum less head.
GERSTEL Cryogenic Trapping System (CTS2)
Maestro software (1.4.20.6/3.5) – Integrated.
Agilent MSD Chemstation E02.02 1431

Figure 1: – Analytical solution for LVI analysis.

Methodology

Commercially available solutions of explosives, Nitroglycerine (NG), Dinitrotoluene (2,4-DNT), Trinitrotoluene (2,4,6-TNT), Pentaerithyritotol Tetranitrate (PETN) and Research Department Explosive (RDX) were kindly donated. Anthracene-d10 was purchased from Sigma-Aldrich (Dorset, UK). Dichloromethane (DCM) (Pesticide Residue Grade), 2-propanol (LC-MS Chromasolv Grade) were purchased from Sigma-Aldrich (Dorset, UK).

Primary stock standards were prepared for all explosives by diluting the appropriate amount of primary standard in either DCM or IPA to form 10 ng/µl single component standards. These standards were then further diluted with DCM or IPA to obtain combined working standard solutions of lower concentrations. The anthracene-d10 solution was diluted in DCM or IPA to give a 1 ng/µl solution; this was further diluted to give a 100 pg/µl solution. This solution was used as an internal standard during calibration and was spiked into all calibrants to give a 10 pg/µl final concentration. All standard solutions were stored at – 18 °C. A five point calibration in IPA was prepared. Calibration was performed in IPA only, from 10, 25, 50, 75 and 100 pg/µl.

Calibration standards were analyzed on an Agilent 7890A gas chromatograph (GC) coupled to an Agilent 5975C MSD.

A CIS 4 programmed temperature vaporization (PTV) inlet, with Universal Peltier Cooling (UPC) GERSTEL (Mulheim, Germany) was used as the injection port and speed programmed injections were performed using the MPS.

Injection speed was optimized using the GERSTEL LVI calculator. For LVI, speed programmed introduction into the liner must be used as the solvent must be removed or vented in the gas phase. If the injection speed is too high, liquid solvent with analytes will simply channel through the liner packing and exit through the split vent. In this scenario an injection of 100 µl could take over 10 minutes, depending on the solvent.

Equal volumes of different solvents result in widely different vapour volumes, requiring very different introduction rates for successful venting of different solvents. When this speed is correctly optimized the solvent will be selectively removed and analytes of interest concentrated in the liner for transfer to the column. Evaporation rate and therefore injection speed is based upon CIS initial temperature, total flow during solvent vent and the vent pressure.

The inlet was operated in solvent vent mode and lined with a deactivated baffled inlet liner. GERSTEL (Mulheim, Germany). The inlet was operated in traditional solvent vent mode with an initial temperature of 20 °C. These conditions were maintained during sample injection (100 µl) and for 1.35 and 0.35 minutes after start for DCM and IPA respectively, at which point the temperature was ramped at 12 °C/sec to 200 °C and held for 5 minutes. During 100 µl injection solvent vent conditions, the vent pressure was 1.94 psi with a vent flow of 100 ml/min.

A CTS2 was employed for peak refocusing. Initial temperature was 0 °C. Following CIS desorption and CTS2 refocusing the CTS2 was ramped at 20

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°C/sec to 200 °C and held for 5 minutes. It was found that lower injection volumes (10 µl) did not require CTS2 refocusing (data not shown).

Analytes were separated on a J&W DB-5MS (15 m x 0.25 mm x 0.25 µm film thickness) column using 1.5 ml/min ultra-high purity helium under constant flow conditions. The oven temperature program was as follows; 60 °C held for 2.0 min then raised at 12.5 °C/min to 210 °C and held for 2 min (total run time 16 min). The transfer line temperature was maintained at 250 °C.

Mass spectrometric ionization was accomplished in electron ionization (EI) mode with a source temperature of 230 °C. The MSD was operated in single ion monitoring (SIM) mode.

In order to identify the most suitable ions for SIM acquisition, full scan spectra were gathered for each compound to identify quantifier (Q) and qualifier (q) ions. (See Table 1). Dwell time during SIM acquisition was 25 msecs.

Compound	Quantifier Ion	Qualifier Ion	R.T
NG	46.0	76.0	6.79
2,4-DNT	165.0	89.0	8.31
2,4,6-TNT	210.0	89.0	9.89
PETN	46.0	76.0	10.65
RDX	46.0	120.0	11.24
Anthracene-d10	188.1	160.2	10.15

Table 1:- SIM method parameters.

Results

Calibration

Quantitative determination of the analytes in IPA was achieved using an internal standard calibration. Calibration curves were comprised of five points. A calibration curve of the relative response of each analyte versus relative concentration ratio of the analyte to anthracene-d10 was generated from these standards.

Calibration curves were generated from average replicate analysis (n=3) of all standard points plotted on the same graph.

Calibration correlation coefficients of 0.998 and above were achieved for all analytes. Example calibration plots for NG and RDX are shown below (See Figure 2.) Calibration curve fit was quadratic for all analytes.

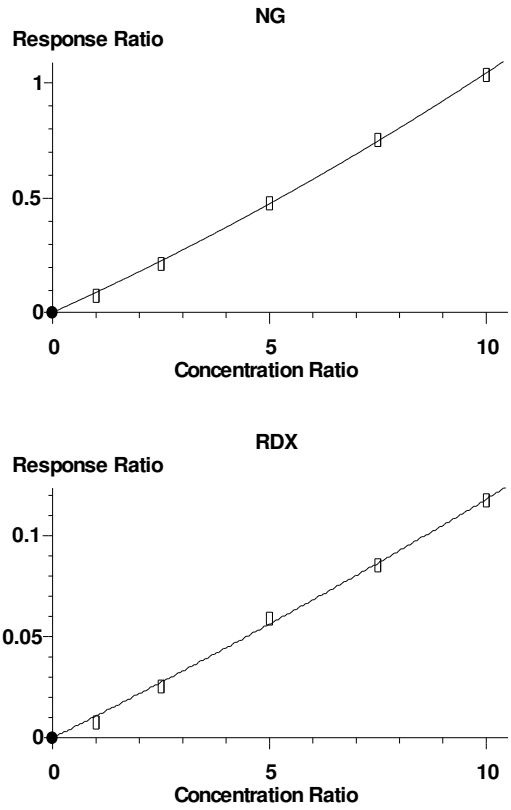

Figure 2 – Calibration plots for NG and RDX

Precision

Replicate analysis (n=3) of a 10 pg/µl standard in IPA yielded % RSD's for all components between 3.6 and 8.6 See Table 2.

Analyte	Average	SD	% RSD
Anthracene-d10	966362	43607	4.5
NG	71705	4007	5.6
DNT	81258	7188	8.6
TNT	8792	742	8.5
TNT 2nd	5427	352	6.5
PETN	19534	765	3.9
RDX	7628	271	3.6

Table 2:- Precision data from triplicate analysis of a 100 µl 10 pg/µl LVI.

Analyte Protectants

During development of this method analytes were diluted and injected in DCM as it is one of only a few solvents in which all components are soluble. Due to the limited differential in boiling point between DCM (39.6 °C) and the lowest boiling analyte (Nitroglycerine (50.0 °C)) of only 10 °C, the use of analyte protectants (keeper) was investigated, to eliminate some of the losses that would occur during lengthy solvent vent times of a speed

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programmed injection of this volume. Results from these experiments proved that the use of a keeper (20 µl of decane per ml of sample) gave increased abundances' for the same amount of analyte injected on column (1 ng) (data not shown). During this development injection volume was only 10 µl so the overall effect of the keeper during a 100 µl DCM injection would increase. % RSD's with the use of a keeper also were better ranging from 1.36 to 7.85 versus 4.56 to 7.58 without keeper.

GC Oven Program

Investigations were made, following selection of the 15 m DB-5MS column into the effect of oven ramping and residence time on the column. It was found that a steep thermal gradient above 12.5 °C/min caused analyte losses, in the instance of using an oven ramp of 25 °C/min PETN and RDX underwent complete degradation.

CIS Liner Selection

CIS liner selection was careful considered during development of this method, the use of a packed liner would allow for slightly quicker speed programmed injection, potentially minimising analytes losses during solvent vent. The use of deactivated packed liners containing glass wool or glass beads was evaluated. In all experiments significantly reduced analyte abundances were observed when using packed liners it was decided to proceed with a deactivated baffled liner for future work.

CTS2 Usage

Following development of a method for injecting 10 µl of a DCM standard, work was started to increase this injection volume to 100 µl. It was soon noted that an injection of a 100 µl caused severe problems with analyte peak shapes. Optimisation work was carried out to try to rectify this issue, with longer solvent vent time, increased vent flows, slower speed programmed injections and lower oven starting temperatures for refocusing. In all cases these options failed to resolve the peak shape issue. Using a CTS2 at 0 °C to refocus analytes following desorption from the CIS resolved the issue and was incorporated into the method. Various temperatures for refocusing were assessed and it was found that 0 °C gave optimum results. See Figure 3.

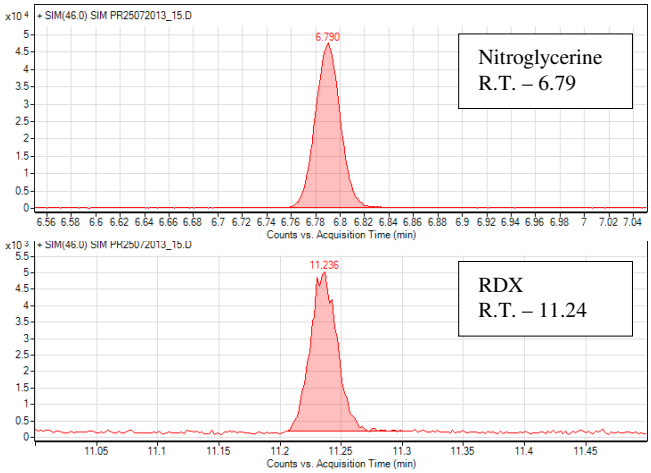

Figure 3 :- Peak shapes for NG and RDX from a 100 µl IPA injection using the CTS2 for refocusing.

Switching from DCM to IPA

Following successful development of two methods (10 and 100 µl injection) in DCM the use of IPA for injection solvent was evaluated. IPA was chosen as it has been found that it is not particularly aggressive towards the plastics from which the explosives are extracted from.

Work was completed to assess changing the speed programmed injection due to the change in solvent, revaluating solvent vent time and vent flow. Following this development vent flow was maintained at 100 ml/min but vent time was decreased to 0.35 minutes.

Additionally the speed programmed injection was reduced from 0.34 µl/sec when using DCM to 0.18 µl/sec when injecting IPA due to the reduced evaporation rate under the same CIS conditions.

The use of decane keeper was also revaluated due to the elevated boiling point of IPA (82.6 °C) compared to DCM.

Conclusions

This application note details the excellent performance of the GERSTEL CIS4 inlet when used in solvent vent mode for large volume injections (100 µl) in challenging application scenarios. Cooling using the UPC gives excellent temperature stability at 20 °C, which is critical when performing LVI as small changes in inlet temperature can dramatically effect solvent evaporation rates and precision as a consequence.

Excellent precision was observed for 100 µl injections in IPA and DCM. The generated correlation coefficients of > 0.998 indicate that the method is suitable for fully quantitative analysis.

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