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Published December 2023

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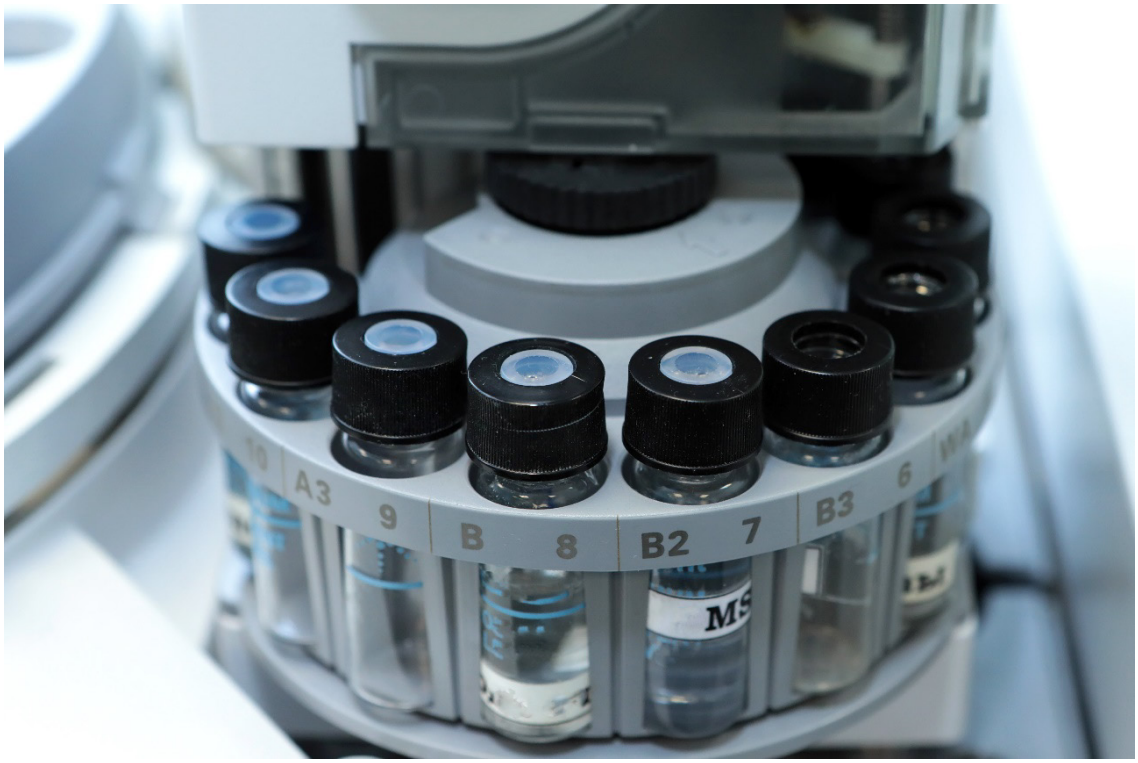
The Forensic Laboratory Needs Technology Working Group (FLN-TWG), formed by the National Institute of Justice (NIJ) in partnership with the Forensic Technology Center of Excellence (FTCOE) at RTI International, created this document in support of NIJ's mission to improve knowledge and understanding of federal, state, local, and tribal forensic science service providers' (FSSPs') technology needs.



TECHNICAL NOTE
Gas Chromatography-Vacuum Ultraviolet Spectroscopy (GC-VUV)

Introduction

Gas chromatography–vacuum ultraviolet spectroscopy (GC-VUV) was first introduced in 2014[1] as a universal separation and detection technique. It combines chromatographic separation of volatile and semivolatile analytes with spectroscopic detection based on the absorption of light in the UV and VUV regions, with the VUV region being defined as below 200 nm.[2] GC-VUV provides enhanced positional isomer differentiation capabilities compared with gas chromatography–electron ionization mass–spectrometry (GC-EI-MS), particularly for isomers differing in substitution on a benzene ring and certain instances pertaining to aliphatic substitution. VUV detection is considered a universal detection technique in seized drug casework because nearly every chemical species absorbs strongly in the VUV region based on electronic transitions of electrons in single bonds ($\sigma \rightarrow \sigma^*$) and double bonds ($\pi \rightarrow \pi^*$), which provide unique absorption signatures.[1, 2] However, because of the nearly universal absorption in the VUV region, VUV spectra must be collected in a flow cell purged with a non-absorbing background gas, such as diatomic nitrogen.[3] GC-VUV spectra contain more spectral features than liquid-phase UV spectra because of the removal of the solvent, which masks vibrational features and imposes a cutoff at approximately 195 nm that eliminates useful information in the VUV region.[4] GC-VUV provides qualitative analyte identification through the comparison of a VUV spectrum to a VUV spectral library and quantitative determinations governed by the Beer-Lambert Law.[1, 2, 5]

A VUV detector can be coupled with any standard GC instrument, including fast GC applications, due to a data acquisition rate of up to 100 Hz.[1] As a sample elutes from the GC column, it enters the VUV detector through a heated transfer line, where a make-up flow or purge gas is used to sweep the sample into the flow cell. The sample is then irradiated with broad band light emitted from a deuterium lamp. The emitted light is passed through the flow cell with magnesium fluoride windows before reaching a holographic grating for diffraction onto a charge-coupled device detector.[2, 3] The absorbance is determined by taking the log ratio of the measured intensity of the initial lamp output in the absence of sample (I_o) divided by the measured intensity when analyte is present in the flow cell and absorbing light (I). Although GC-VUV is not specifically addressed in either the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommendations[6] or ASTM E2329-17 Standard Practice for Identification of Seized Drugs,[7] GC is recognized as a Category B technique and UV spectroscopy is recognized as a Category B and Category C technique, respectively. It should be noted that because VUV spectra contain more spectral features than liquid-phase UV spectra, VUV detection should probably be characterized as at least a Category B technique.

Capabilities

The main benefits of GC-VUV analysis relative to the GC-EI-MS technique traditionally used for seized drug analysis include enhanced differentiation of closely related chemical species[8-13] and simpler spectral deconvolution.[8, 10-12, 14] VUV spectra contain relatively small but significant variations that allow for the differentiation of closely related chemical species. This is due to the high repeatability of VUV spectra and VUV library spectral matches.[9, 11] In fact, VUV library spectral comparisons have been demonstrated to produce deviations in library match scores less than 0.1% across two months of data collection,[9] which has led to a proposed 0.998 library match score as a potential alarm threshold for the presence of mixtures when compared against pure reference spectra.[10] Even in the absence of the correct compound in the reference VUV library, the returned matches are likely to share structural features with the unknown compound that could be used to guide further structural elucidation.[10] VUV detection appears to be particularly useful for differentiating aromatic ring positional isomers,[9] which have become increasingly prevalent in seized drug casework. In addition, this detection technique can distinguish positional isomers differing in aliphatic substitution.[11] Likewise, VUV detection provides some differentiation capabilities based on the length of the alkyl substitution.[15] In terms of forensically relevant compounds, GC-VUV has been demonstrated for the analysis of phenethylamines,[12, 13] synthetic cathinones,[8-10] fentanyl analogs,[11] and classical seized drugs such as cocaine and heroin.[16,17] GC-VUV also provides enhanced co-elution deconvolution capabilities because the measured absorption spectrum of co-eluting species is simply the sum of their absorption features scaled according to their relative abundances. This means that co-eluting spectra can be deconvoluted using a linear combination of scaled reference spectra to project the contribution of each individual analyte to the co-elution spectrum.[14] The ease with which GC-VUV spectra can be deconvoluted means that GC-VUV does not require extensive chromatographic method development, parameter optimization, and instrument tuning.[9] This makes the limits of VUV detection superior to what is obtainable using gas-phase infrared detection because of increased absorption cross-sections in both VUV and UV regions leading to low nanogram on column limits of detection.[2,3] **Exhibit 1** summarizes the cost and analysis time of GC-VUV instrumentation.

Exhibit 1. Summary of GC-VUV cost and analysis time considerations.

Initial Cost	Cost/ Analysis	Analysis Time	Cost-saving Considerations
~$39,000–$99,000 (VUV detector only) ~$75,000—$150,000 for the complete GC-VUV system	See current laboratory GC-EI-MS costs	See current laboratory GC-EI-MS time	Purchase VGA-100 or VGA-101 detector separately rather than combined with GC vendor Purchase the LUMA detector $39,000 (4 channel) and $45,000 (12 channel), rather than the VGA series detector

Limitations

The limitations of GC-VUV instrumentation include the relative lack of structural information,[5] issues with disparate co-eluting mixtures,[10] and relatively high limits of detection,[2] although this is not typically an issue for seized drug casework. Although VUV spectra are highly repeatable and contain both subtle and discernible differences that allow for analyte identification through comparison to a reference library,[9, 11] VUV spectra only provide limited structural information about the analyte.[5] This limitation must be considered when a forensic laboratory assesses where GC-VUV would fit into their typical analytical scheme as defined by SWGDRUG[6] and ASTM E2329-17.[7] However, VUV detection can distinguish between analytes that are difficult to differentiate with EI-MS, which should also be considered when determining how GC-VUV would fit into a laboratory's analytical scheme.[8-9, 11-12] GC-VUV allows for simple spectral deconvolution of co-eluting analytes, which makes GC-VUV a more user-friendly instrument from a method development perspective.[9] However, there are potential issues with the spectral deconvolution of co-eluting analytes when there is a great disparity between the major and minor contributors. For example, the differentiation of 3-methylmethcathinone (3-MMC) and 4-MMC became quite difficult at a ratio of 9:1 between the major and minor contributors because of the high spectral similarity between these two closely related compounds.[10] In comparison, ratios as disparate as 1:99 have been demonstrated to allow for successful spectral deconvolution of components that contain different VUV spectral features, such as 1,4-dimethylnaphthalene (1,4-DMN) and 2,3-DMN.[18] Recently, 50:50 mixtures of dimethoxyamphetamine isomers were successfully deconvoluted even with highly similar VUV spectra and significant co-elution.[12] Finally, the limit of detection for most reported GC-VUV applications is on the order of low µg/mL,[8, 11-13] which may not be appropriate for all seized drug applications. According to a review of GC-VUV literature by Santos and Schug, the typical reported detection limit for VUV detection is approximately 20 times higher than EI-MS detection.[2]

Installation needs

The VGA-100 and VGA-101 detectors can be coupled with any commercial GC instrumentation either in place of, or combined with, the existing detector. The VUV detector comes with all required transfer line connectors and can be mounted on either side of the GC. The VUV detector is powered by a single 100–240 VAC power source and the VUVision software required for data collection and analysis is installed on the same computer (existing or otherwise) as the GC software. In addition to the existing GC carrier gas, the VGA-100 and VGA-101 detectors require an additional ultra-pure nitrogen cylinder, clean dry air gas cylinder, and corresponding triple gas (e.g., oxygen, moisture, and hydrocarbon) inline filters. The LUMA detectors can presently only be coupled with Agilent GC instruments. This detector works with existing laboratory chromatography data systems. All gases, including the purge gas and make-up gas, are supplied through the auxiliary electronic pressure control on the GC. For this latter detector there are no pneumatic components, so no clean dry air gas is required. The LUMA outputs both analog and digital signals. The digital data can be acquired either using a common data service driver or the LUMA OS Console application run on the computer. The analog outputs on the LUMA can be used with analog boards on the GC to collect data in the common data service. If digital data are used, there is no need to have analog boards in place.

Vendor considerations

At this time, the only commercially available VUV detector vendor is VUV Analytics (Cedar Park, TX), who manufacture the VGA-100, VGA-101, and the LUMA multichannel VUV detectors. The VGA model detectors can be coupled with any standard GC instrument through a punch-out in the GC oven casing. The VGA-100 contains a 10 cm path length (80 µL flow cell) and covers the 120–240 nm wavelength range. The VGA-101 detector provides an increased wavelength range (120–430 nm), a smaller flow cell (40 µL), and higher heating capabilities for the transfer line and flow cell, but the higher temperatures may lead to sample degradation for forensically relevant samples. VUV Analytics have agreements with all major GC manufacturers, so that their technology can be purchased as a standalone detector to be coupled with an existing system or purchased in combination with new GC instrumentation.

In comparison to the VGA model detectors, the LUMA flow cell is 10 cm long with an internal diameter of 0.76 mm and has a 45 µL volume. The LUMA detectors install directly on

top of existing Agilent 6890, 7890, and 8890 GCs. Instrumental control and data handling are accomplished using VUVision software. For the LUMA detectors, both 4- and 12-channel models are available, with each channel consisting of a unique wavelength range from 120–430 nm as demonstrated in Exhibit 2. Using the LUMA multichannel detector, seized drugs can be identified using both GC retention time and one or more peak area ratios derived from detection at unique wavelength ranges. For a given analyte, the peak area ratios are proportional to their extinction coefficients over a selected range. Baker et al. demonstrated the use of relative retention times and dual wavelength absorbance ratios (proportional to peak area ratios) to identify seized drugs, and they have been used for both the identification of fentanyl and synthetic cathinone analogues.[19,20,21]

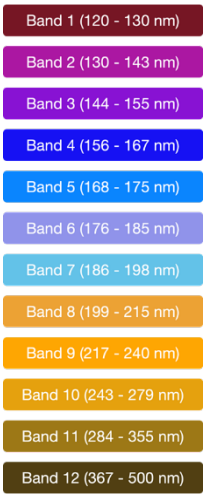


Exhibit 2. Example unique wavelength range for each channel of the 12-channel LUMA detector.

Space requirements

The VGA-100 detector weighs approximately 120 lbs. and requires a physical footprint of 40.8 cm (16.1”) × 34.0 cm (13.4”) x 69.9 cm (27.5”) (height × length × depth) with an additional 12.7 cm (5”) of plumbing hook-up space between the GC and the VUV detector. This detector requires one dedicated 100–240 VAC, 50–60 Hz, 10 A grounded outlet with a power of 700 W and two ultra-high purity gases (99.999% or greater). The nitrogen, helium, or argon system gas, which controls the flow of analyte through the flow cell and minimizes background absorption, requires an input pressure of 60–90 psi. The clean dry air gas (zero grade or better) that controls the shutter and pneumatically operated valves requires an input pressure of 70–90 psi. The use of high-quality oxygen, moisture, and hydrocarbon traps immediately after the main tank pressure regulator is highly recommended by VUV Analytics. The only difference between the requirements for the VGA-100 and VGA-101 is the power requirement of 1,200 W. The LUMA detector, which is installed on top of the GC, weighs approximately 20 lbs., and the unit is 15.2 cm (6”) wide, 23.5 cm (9.25”) tall, and 51 cm (20”) deep. The power supply unit is 30.5 cm (12”) × 15.2 cm (6”) × 10.8 cm (4.25”), and requires one dedicated 100–240 VAC, 50–60 Hz, and 10 A grounded outlet. The LUMA detector also requires an ultra-high purity gas (99.999% or greater) for a system purge gas and a make-up gas. Both these gases are controlled by AUX EPCs on the GC. The gases can be nitrogen, helium, or argon. The use of high-quality oxygen, moisture, and hydrocarbon traps immediately after the main tank pressure regulator is highly recommended.

User training and skill level

There is a 3-day installation and training session that comes with the purchase of a VUV detector from VUV Analytics. Installation for the VGA-100 or VGA-101 detector typically only takes about half a day, with the remainder of the time being dedicated to training. In contrast, the LUMA detectors are user installable in less than 1 hour. VUV Analytics also offers quarterly training sessions for an additional cost at Cedar Park, TX. All necessary skillsets for GC-EI-MS or GC-FID sample preparation, maintenance, and interpretation are required with the only new skillsets that need to be developed being the use of the VUVision software and VUV troubleshooting.

Implementation needs

The biggest inhibitors to the implementation of GC-VUV into forensic casework are the limited amount of peer-reviewed literature, including method validation studies, somewhat limited VUV spectral libraries, and buy-in from the forensic community. Only a few forensic laboratories have adopted this technology and are in the process of method validation before incorporation into routine casework analysis (see **Exhibit 3**).

Validated methods

One of the factors limiting the incorporation of GC-VUV technology into routine forensic casework is the lack of peer-reviewed literature involving GC-VUV performance characteristics for forensically relevant compounds. Two examples are the works of Buchalter et al.[11] and Roberson et al.,[13] which explored the application of GC-VUV to fentanyl analogues and phenethylamines. Buchalter et al. explored the use of GC with tandem cold EI-MS detection and VUV detection for the analysis of 24 fentanyl analogues. This work demonstrated that VUV provides excellent linearity (R^2 ≥ 0.999) for most analytes over two orders of magnitude with limits of detection of 260–585 ng/mL. This study also examined the repeatability of the GC-VUV retention time, peak area, and library match scores, which resulted in relative standard deviations (%RSD) of 0.1%–0.14% (n = 5), 0.4%–10.1% (n = 5) (≥ 3.8% at levels near the limit of quantitation), and ≤ 0.34% (n = 5), respectively. The analysis of seven simulated casework samples resulted in recoveries of 91.2%–115% but revealed the limitation of VUV detection for trace and ultra-trace analysis. In comparison, Roberson et al. examined eight phenethylamines and determined the limit of detection to be 10 ng on column and a linear range of 10 to 1000 µg/mL with a coefficient of determination (R^2) of 0.9971.[13] Accuracy and precision were assessed for a trifluoroacetic anhydride derivative of n-methylphenethylamine and determined to be −0.26% and 0.62% RSD, respectively. However, when authentic samples were analyzed, there were issues with the identification of unknown compounds that were not present in the VUV library. In a follow-up study, response surface modeling was used to perform a statistical optimization of the VUV method parameters and identified that the carrier gas flow rate was the most important factor for the analysis of cocaine, heroin, fentanyl, methamphetamine, phencyclidine (PCP), lorazepam, and HU-210.[16] With the optimized VUV parameters, limits of detection of 1.1–38 ng on column were determined, which is comparable to existing GC-EI-MS limits of detection in full scan mode.[16]

Searchable libraries

There is a searchable library that comes with the VUV detector that contains at least 1,000 organic compounds,[8] but currently, only approximately 150 are forensically relevant (personal communication with VUV Analytics). However, because VUV spectra are not specific to an instrumental setup, the VUV spectra collected on any GC-VUV system can be loaded into the user's library. Several research groups (see **Literature**) are collecting data on forensically relevant compounds that could be incorporated into the existing library should a laboratory pursue this option. New analytical reference compounds can also be analyzed and added directly to the user's VUV library at any time.

Data interpretation

The VUVision software comes with a searchable library and spectral deconvolution, known as time interval deconvolution, based on linear regression of scaled reference spectra from the searchable library. In addition, even if an unknown compound is not present in the library, because VUV spectra provide class-specific information, the highest hits from the library are likely to be closely related to the unknown compound.

Reporting and testimony

The reporting and testimony associated with GC-VUV instrumentation will parallel traditional GC-EI-MS reporting and testimony. The unknown will still have a retention time, and the generated spectrum will still be used for identification based on the combination of a spectral library search and the analyst's interpretation. Given the currently limited implementation of this technique into forensic casework, there are no examples of common testimony language to report at this time.

Consumables

The only consumable product for a VUV detector is the deuterium lamp. The expected lifetime for the deuterium lamp is 6 to 12 months depending on the frequency of use. A replacement lamp assembly costs approximately $2,500. Additional consumables associated with GC-VUV instrumentation are the same as those used for GC upkeep and maintenance. Laboratories should see their current GC consumables expenditures for a cost estimate.

Early adopting laboratories

Exhibit 3. Point of contact information from early adopting laboratories that have started the implementation of GC-VUV into casework. This list may not be exhaustive but is intended to highlight points of contact for those interested in GC-VUV implementation.

Laboratory	Point of Contact	Email	Phone Number
Westchester County NY	John T. Clark	JTC3@Westchestergov.com	(914) 231-1630
Dutch Customs Laboratory, Amsterdam	Behrad Ghavim	b.ghavim@douane.nl	+31 (0)6 15 87 47 59

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The FTCOE, led by RTI International, is supported through a Cooperative Agreement from the National Institute of Justice (15PNIJ-21-GK-02192-MUMU), Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this report are those of the author(s) and do not necessarily reflect those of the U.S. Department of Justice. Information provided herein is intended to be objective and is based on data collected during primary and secondary research efforts available at the time this report was written.

Suggested Citation

Davidson, J. T., & Lurie, I. (2023, December). *Gas chromatography-vacuum ultraviolet spectroscopy (GC-VUV)*. Research Triangle Park, NC: RTI International.