# Separation of Oxymorphone and Oxycodone Hydroxyl-imino Tri-methy Silyl Derivatives Using an Agilent Fast Toxicology Analyzer and an Agilent J&W DB-35ms Ultra Inert Capillary GC Column

Application

Forensics


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## Abstract

Oxymorphone and oxycodone are semi synthetic opioids used primarily to manage moderate to severe pain. Within the last few years, several previously unavailable oxymorphone oral dosage forms have been introduced to the marketplace. Unfortunately, these newer dosage forms are finding their way into the hands of abusers with the potential for lethal overdose as a result.

Hydroxyl-imino tri-methy silyl derivatives of these drugs of abuse do not resolve either chromatographically or mass spectrally on the 5 % phenyl columns typically used for this type of GC/MS analysis. A successful chromatographic separation of oxymorphone and oxycodone hydroxyl-imino tri-methy silyl derivatives is demonstrated on an Agilent J&W DB-35ms Ultra Inert (UI) capillary GC column.


## Introduction

These new formulations are designed for immediate release to manage breakthrough pain and extended release to maintain a steady pain relief effect over longer periods of time. [1]

Unfortunately, drug diversion and illicit use of these drugs is common as drug abusers chase the euphoric side effect that these powerful narcotics produce. [2] The recently introduced oxymorphone oral dosage forms are finding their way into the hands of drug abusers where deaths by overdose can and have taken place. Drug abusers are circumventing the extended release properties of these tablets by snorting, chewing, or taking them with alcohol. These are very dangerous practices as oxymorphone euphoric effect is modest in comparison to similar opioids and its bioavailability varies widely. By chasing the euphoric kick opiates provide in the

context of unpredictable dosage delivery, drug abusers can easily obtain a lethal dose.

Analysis of these drugs of abuse in matrices such as whole blood can be challenging, particularly at trace levels. The Fast Toxicology Analyzer coupled with a highly inert Agilent J&W DB-35ms Ultra Inert (UI) capillary GC column meet the challenge of delivering accurate results in difficult matrices quickly [3]. The selectivity and exceptional inertness of the Agilent J&W DB-35ms UI column proves essential to this separation, as the derivatives of interest co-elute using a 5% phenyl column. The molecular ion profiles for these derivatives also share a common ion and their selective ion profiles had significant overlap with each other and their respective deuterated analogs [4].

## Experimental

*Table 1. Instrument Conditions Fast Screening Agilent J&W DB35ms UI*

| | |
|---|---|
| Carrier: | Helium fixed pressure 35.0 psi |
| Inlet: | Splitless 1 µl 280 °C, total flow 56.4 mL/min, 3 mL/min switched septum purge, gas saver off, 50 mL/min after 0.4 min |
| Sample: | Agilent GC/MS Toxicology Checkout Mixture (Agilent p/n 5190-0471) |
| Inlet Liner: | Dual taper deactivated (Agilent p/n 5181-3315) |
| Column: | Agilent J&W DB-35ms UI 15 m × 0.25 mm, 0.25 µm (Agilent p/n 122-3812UI) 35 psi constant pressure mode |
| Backflush: | Post run: 1 min. 1 psi inlet, 75 psi aux EPC |
| Oven: | 100 °C (0.25 min) to 345 °C (40 °C/min, 2.25 min hold) |
| MSD: | Transfer line 300 °C, source 300 °C, quadrapole 180 °C scan mode |
| NPD: | Blos bead 300 °C $H_2$ 3 mL/min, 60 mL/min air, 11 mL/min makeup and col flow |
| CFT Device: | 2-Way splitter with solvent venting between MSD and NPD |

*Table 2. Instrument Conditions Quant 5% Phenyl Colums*

| | |
|---|---|
| Carrier: | Helium constant flow 1.0 mL/min |
| Inlet: | Splitless 1 µl 280 °C, total flow 56.4 mL/min, 3 mL/min switched septum purge, gas saver off, 50 mL/min after 0.4 min |
| Sample: | Whole blood extract |
| Inlet Liner: | Dual taper deactivated (Agilent p/n 5181-3315) |
| Column: | Agilent J&W DB-35ms UI 15 m × 0.25 mm, 0.25 µm (Agilent p/n 122-3812UI) 35 psi constant pressure mode |
| Back-flush: | Post run: 1 min. 1 psi inlet, 75 psi aux EPC |
| Oven: | 100 °C (1 min) to 325 °C (10 °C/min, 5 min hold) |
| MSD: | transfer line 300 °C, source 300 °C, quadrupole 180 °C scan mode |
| NPD: | Blos bead 300 °C $H_2$ 3 mL/min, 60 mL/min air, 11 mL/min makeup and col flow |
| CFT Device: | 2-Way splitter with solvent venting between MSD and NPD |

*Table 3. Instrument Conditions Quant Agilent J&W DB-35ms UI*

| | |
|---|---|
| Carrier: | Helium constant flow 1.0 mL/min |
| Inlet: | Splitless 1 µL 280 °C, total flow 56.4 mL/min, 3 mL/min switched septum purge, gas saver off, 50 mL/min after 0.4 min |
| Sample: | Whole blood extract |
| Inlet Liner: | Dual taper deactivated (Agilent p/n 5181-3315) |
| Column: | Agilent J&W DB-35ms UI 15 m × 0.25 mm, 0.25 µm (Agilent p/n 122-3812UI) 35 psi constant pressure mode |
| Back-flush: | Post run: 1 min. 1 psi inlet, 75 psi aux EPC |
| Oven: | 100 °C (1 min) to 345 °C (10 °C/min, 9 min hold) |
| MSD: | Transfer line 300 °C, source 300 °C, quadrupole 180 °C scan mode |
| NPD: | Blos bead 300 °C $H_2$ 3 mL/min, 60 mL/min air, 11 mL/min makeup and col flow |
| CFT Device: | 2-Way splitter with solvent venting between MSD and NPD |

*Table 4. Ions of Interest*

| **Oxycodone hydroxyl-imino tri-methyl silyl derivatives** | **Oxymorphone hydroxyl-imino tri-methyl silyl derivatives** |
|---|---|
| Principal ions OCOD | Principal ions OMOR |
| 459 analyte (common ion) | 459 analyte (common ion) |
| 474 analyte | 533 analyte |
| 465 d6 analog | 462 d3 analog |
| 480 analog | 536 d3 analog |

## Sample Preparation

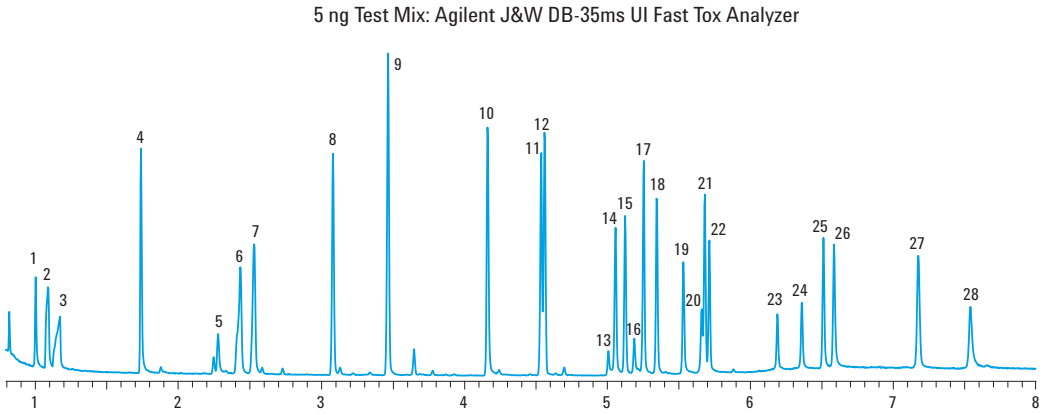
A GC/MS Toxicology Checkout Mixture (Agilent p/n 5190-0471) containing 28 drugs of abuse was transferred to sample vials and used as received. Using this mixture and a 1-µL injection volume delivers a nominal on column loading of 5 ng/component. Proadifen (SKF-525A), which is used as the retention time locking compound for the Fast Toxicology Analyzer, is contained in this mix.

Known concentrations of oxycodone, d6-oxycodone, oxymorphone, and d3-oxymorphone were spiked into UTAK whole blood. The samples underwent protein precipitation using methanol and acetonitrile. They were centrifuged, and the supernatant pH was adjusted to pH 4.5 with acetate buffer. Keto-opioids are subject to tautomerism depending upon matrix conditions so they must undergo derivatization to avoid recovery issues. Ten percent hydroxylamine was added and the samples were heated to 60 °C in a dry heat block for 30 min to complete the oxime derivatization. Once cooled, pH 6.0 phosphate buffer was added for solid phase extraction (SPE.) SPE was carried out on positive pressure manifolds using copolymerized mix mode SCX/SPE columns. Samples were then derivatized using BSTFA at 90 °C for 30 min to yield tri-methylsilyl derivatives for injection onto the GCMS.

## Results and Discussion

Figure 1 shows the separation of 28 underivatized drugs of abuse on an Agilent J&W DB-35ms UI column with a nominal on-column loading of 5 ng per component. The peak shapes observed for some of these very active analytes, even at this relatively low level, are sharp and symmetrical facilitating good quantification. The check-out mix contains a broad range of basic and acidic drugs from several drug classes and provides an effective tool for quick assessment of column and system performance. In this study the column and system perform well.

Using the Fast Toxicology Analyzer along with retention locked DRS data bases and method translation software, it is a simple process to convert from a fast screening method to a focused quantitative method on the same instrument. In busy forensic toxicology labs a large number of samples can be screened in fast analysis mode and then the system can be switched to higher resolution quantitative analysis on positive only samples. This is the approach shown in this application. Figure 1 illustrates the fast screening method where subsequent figures highlight the quantitative analysis of oxycodone and oxymorhphone derivates first on a 5% phenyl column and then on an Agilent J&W DB-35m UI column.




**Compound List**

| | | | |
|---|---|---|---|
| 1 Amphetamine | 8 Meperidine | 15 Codeine | 22 Flunitrazepam |
| 2 Phentermine | 9 Phencyclidine | 16 Lorazepam | 23 Nitrazepam |
| 3 Methamphetamine | 10 Methadone | 17 Diazepam | 24 Clonazepam |
| 4 Nicotine | 11 Cocaine | 18 Hydrocodone | 25 Alprazolam |
| 5 Methylenedioxyamphetamine(MDA) | 12 SKF-525a (RTL Compound) | 19 Oxycodone | 26 Verapamil |
| 6 Methylenedioxymethamphetamine(MDMA) | 13 Oxazepam | 20 Temazepam | 27 Strychnine |
| 7 Methylenedioxyethylamphetamine | 14 Tetrahydrocannabinol | 21 Diacetylmorphine | 28 Trazodone |

*Figure 1. Example NPD chromatogram of underivatized drugs of abuse 5 ng/component on an Agilent J&W DB-35ms UI column fast screening conditions listed in Table 1. Component number 12 is used for retention time locking in the deconvolution reporting software database.*

An extended method for quantification of drugs of abuse at reduced flow rate with a slower temperature ramp was not successful in resolving oxycodone and oxymorphone hydroxyl imino tri-methyl silyl derivatives on the 5% phenyl column. These derivatives are similar enough to one another that additional column selectivity is required to separate them chromatographically. Unfortunately, there is also significant overlap in the SIM profiles of the deuterated internal standard and analyte ions of interest for separation based on their masses. Figure 2 clearly illustrates the issue of SIM ion overlap. This level of overlap of their SIM ion profiles suggests that accurate quantification of these analytes will be difficult at best and impossible at worst. Another approach is needed.

Shifting the separation to a column with more selectivity for these derivatives provides another approach. Using the selectivity of a mid polarity column was successful for this separation. Figure 3 shows the relevant SIM ions for oxymorphone (OMOR) hydroxyl-imino tri-methyl silyl derivatives along with the resolved common oxycodone (OCOD) shared ion. The selectivity of the Agilent J&W DB-35ms UI column provided the power in this separation to resolve the OMOR and OCOD ions chromatographically.

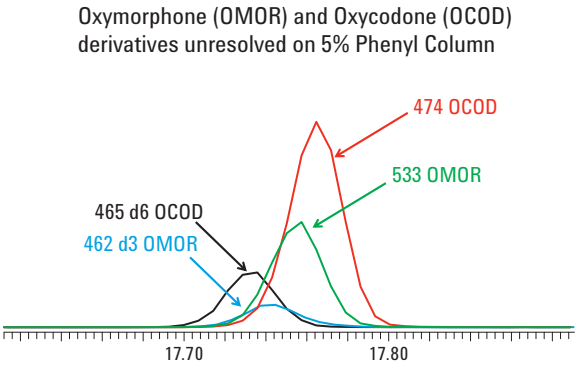



*Figure 2. SIM trace of OMOR and OCOD derivative ions on a 5% phenyl column. The unique analyte ions are shown along with the deuterated internal standard ions. Table 2 lists the GC/MS conditions used for quantitative analysis on the Agilent J&W DB5ms UI column. Table 4 lists the SIM ions.*

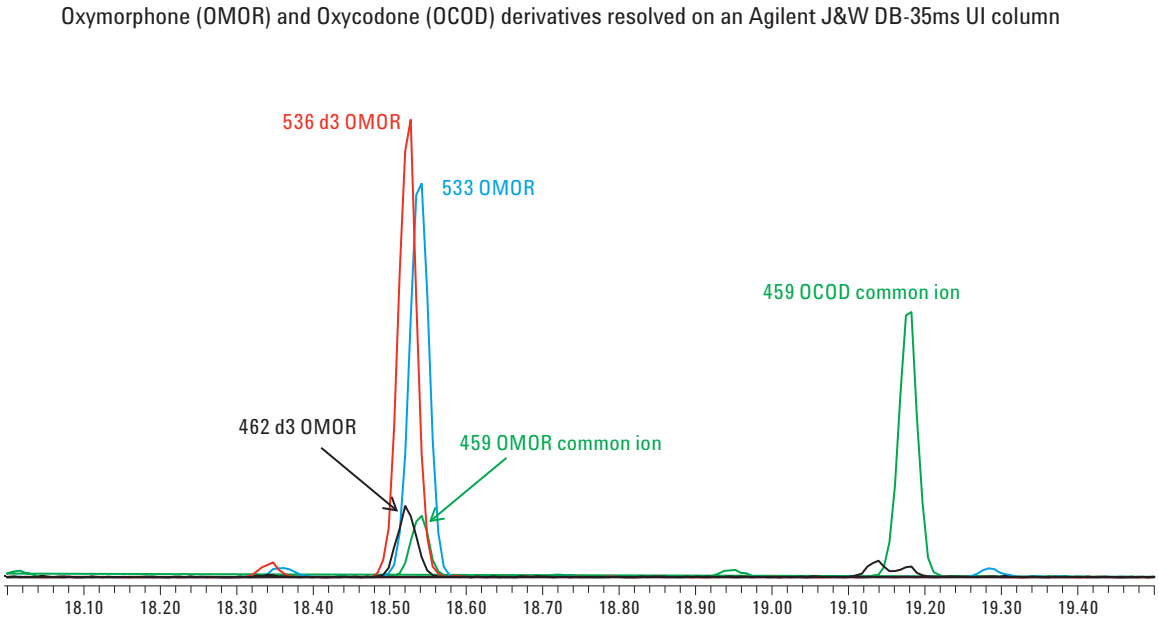



*Figure 3. SIM trace of OMOR and OCOD derivative ions on an Agilent J&W DB-35ms UI. The common analyte ion highlights the peak resolution observed on the Agilent J&W DB-35ms UI. Table 3 lists the GC/MS conditions used for quantitative analysis on the Agilent J&W DB-35ms UI column. Table 4 lists the SIM ions.*

The separation on the Agilent J&W DB-35ms UI column does not interfere with the separation of morphine, d6-morphine, codeine, d6-codeine, hydromorphone, d6-HMOR, hydrocodone, d6-HCOD, 6-MAM and d6-6-MAM. However, 6-MAM did have an overlap with an unknown impurity. A higher concentration sample of 6-MAM was not available to more fully investigate this potential interference.

Figure 4 shows the relevant SIM for oxycodone (OCOD) hydroxyl-imino tri-methyl silyl derivatives along with the resolved common oxymorphone (OMOR) shared ion. The selectivity of the Agilent J&W DB-35ms UI column was sufficient to resolve the OCOD and OMOR analytes from each other chromatographically.

## Conclusions

This application note demonstrates the successful separation of oxycodone and oxymorphone hydroxyl-imino tri-methyl silyl derivatives using an Agilent J&W DB-35ms UI column. This column is a particularly good choice for this application as it offers the selectivity to resolve this challenging pair of analytes along with a high level of inertness. The column's high level of inertness helps to improve peak shapes and assure recovery of low level active analytes such as drugs of abuse.

The selectivity of the Agilent J&W DB-35ms UI column in tandem with the Fast Toxicology Analyzer provides an excellent solution to a current real world issue surfacing in modern forensic toxicology laboratories. Forensic investigators now have a reliable means to resolve oxymorphone and oxycodone hydroxyl-imino tri-methyl silyl derivatives and obtain the answers they need quickly.

The Agilent Fast Analyzer can be configured either with a 5% phenyl or a Agilent J&W DB-35ms UI column for fast screening. The Fast Analyzer can also be used for high resolution quantification methods on the same system. Agilent's MSD Toxicology Analyzer software makes the conversion between screening and quantification straightforward. Screening and quantification can easily be set up to run in the same sequence. Where separation of OMOR and OCOD derivatives are necessary, the Agilent J&W DB-35ms UI column is the preferred choice with the selectivity and inertness to chromatographically resolve this difficult pair.

Oxymorphone (OMOR) and Oxycodone (OCOD) derivatives resolved on Agilent J&W DB-35ms UI Column

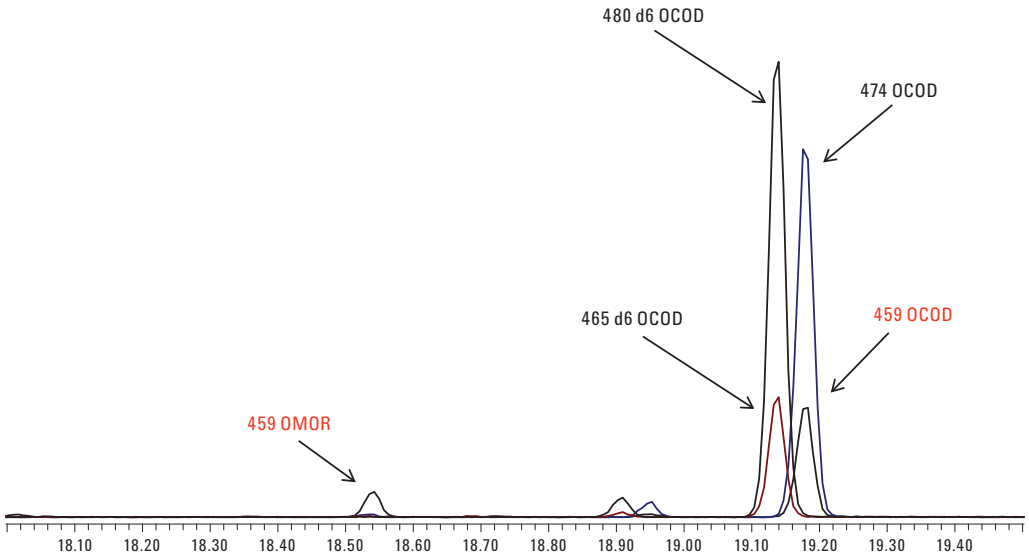



*Figure 4. SIM trace of OCOD and OMOR derivative ions on an Agilent J&W DB-35ms UI column. The common analyte ion illustrates the resolution between the OCOD and OMOR peaks on the Agilent J&W DB-35ms UI column. Table 3 lists the GC/MS conditions used for quantitative analysis on the Agilent J&W DB35ms UI column. Table 4 lists the SIM ions.*

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## Bibliography

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