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Nota de aplicación

Toxicology Screening by UPLC-PDA in Combination with an Extensive Compound Library

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For forensic toxicology use only.

Abstract

This application shows that toxicology screening is greatly improved by transferring a traditional HPLC-UV method to UPLC-UV. The UPLC-UV screening method can be considered cost effective in respect to the initial capital expense when compared to some MS based screening methods. The potential for simpler quantitative or semiquantitative method implementation is also feasible with the system described. In combination with a sixty percent reduction in analytical run time, both the frequency and accuracy of compound identification is increased. The method has been used on a routine basis for more than one year, successfully replacing and enhancing an existing HPLC system.

Introduction

The determination or confirmation of the cause of a suspected poisoning is necessary for the rapid and effective treatment of patients admitted to a hospital after traumatic events such as attempted suicide, accidental poisoning or an adverse drug reaction. Various analytical approaches are possible, which include GC, GC-MS, LC-MS, immunoassay, or LC-UV with the use of UV spectra libraries.

LC-UV is a popular analytical technique as it is often found as standard equipment in many laboratories and patient samples do not routinely require derivatization after extraction, thereby reducing both cost and time. Additionally, the methodology can be semi quantitative. The identification of xenobiotics combines both LC retention time and UV spectra, with a further comparison to a compound library. This type of analytical application is operated routinely in many laboratories with the use of Waters Alliance systems, incorporating a method developed by ToxLab in Paris.[1] Some potential limitations of this particular application relate to chromatographic interferences which impact the UV spectra, leading to inferior library matches. The introduction of UPLC systems has provided dramatic improvements in the resolution of chromatographic peaks, analytical sensitivity and reproducibility of the retention times. Transferring the application from HPLC to UPLC will therefore improve the identification of any unknown compounds in a shorter time.

The compound library that has been created, contains separate UV spectra for 612 compounds along with their relative retention times for the UPLC method as described hereafter. All chromatograms and tables shown in this document are kindly provided, courtesy of Mrs. Camille Chatenay.

Experimental

LC Conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 150 mm, 1.7 µm
Column temp.:	50 °C
Flow rate:	450 µL/min
Mobile phase A:	Acetonitrile/Ammonium acetate buffer 5 mM pH 3.8 (15/85)
Mobile phase B:	Acetonitrile
Detector:	Waters UPLC PDA detector[2]
Wavelength range:	200–400 nm, 40 points/second

Gradient:

Time (min)	%A	%B
0.00	100	0
1.40	100	0

Time (min)	%A	%B
2.80	89.0	11.0
11.00	25.0	75.0
11.20	25.0	75.0
12.00	100	0
15.00	100	0

Sample Preparation:

Sample volume:	1 mL
Extraction:	LLE with Toxitube A*
Internal standard:	20 µL flurazepam at (reference peak) 0.05 mg/mL
Reconstitution:	80 µL volume
Injected volume:	5 µL

* Varian Inc.

Waters ACQUITY UPLC system

Test Mixture:

The test mixture (16 separate compounds), is injected daily to monitor the system performance. It is designed to cover a wide range of the chromatographic run, thereby allowing the operator to identify any areas that might require investigation.

The test mixture is composed of the following compounds: Venlafaxine, Theophylline, Amisulpride, Mirtazapine, Trifluoperazine, Clotiazepam, Imipramine, Diazepam, Clobazam, Prazepam, Oxazepam, Chlordiazepoxide, Caffeine, Paracetamol, Salicylic acid, and Flurazepam.

A chromatogram of the test mixture is shown in Figure 1.

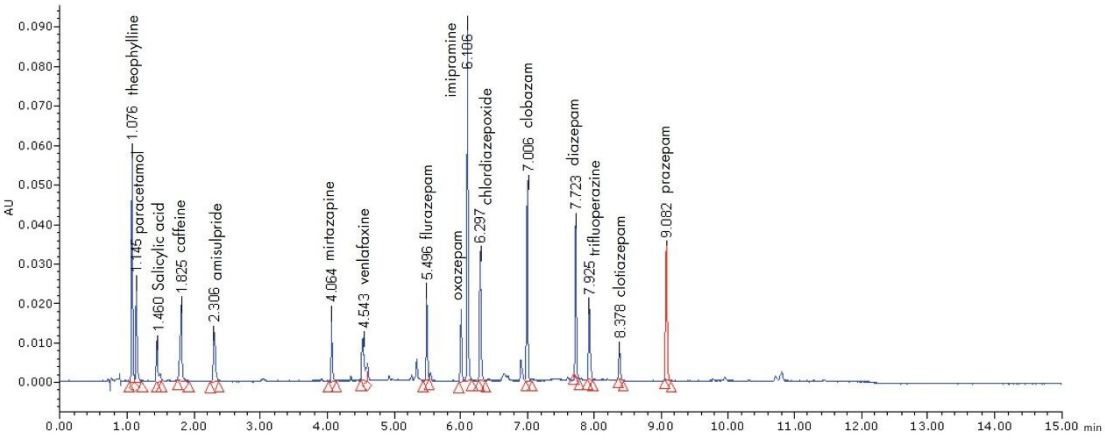

Figure 1. Chromatogram of a test sample mixture with the use of UPLC-UV analysis.

Results and Discussion

The UPLC separation, achieved on a 150 mm column filled with 1.7 µm particles, provides a significantly faster separation (15 minutes vs. 40 minutes), with improved resolution and sensitivity in comparison to a previously established HPLC method. The comparison of samples analyzed using both HPLC and UPLC demonstrates the superior analytical performance of the UPLC methods (Figures 2 and 3).

The identification of a larger number of compounds in a shorter amount of time, are a direct consequence of the improvement in UPLC analytical performance. This has been demonstrated on real samples analyzed using both HPLC and UPLC methods. Figures 2 to 5 illustrate two specific examples of this superior compound identification, where drug metabolites were identified using the UPLC/UV method described.

The use of UPLC allows the identification of more compounds compared to traditional HPLC (Figures 2 and 3). The detailed chromatograms of structurally related compounds (Figures 4 and 5), demonstrate that compound separation is improved by using the Waters ACQUITY UPLC system.

Example 1: Toxicology Screening (Urine)

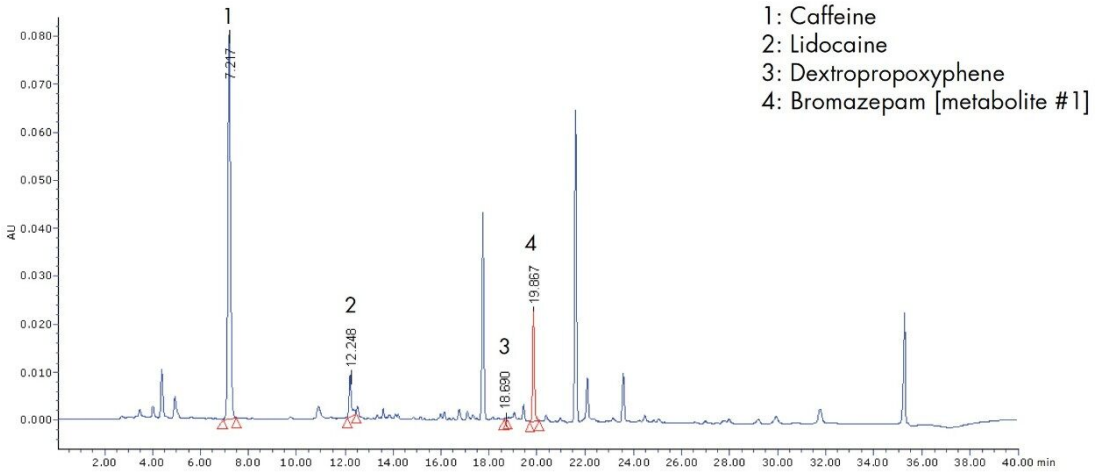

Figure 2. Toxicology screening chromatogram of a urine sample with HPLC/UV analysis.

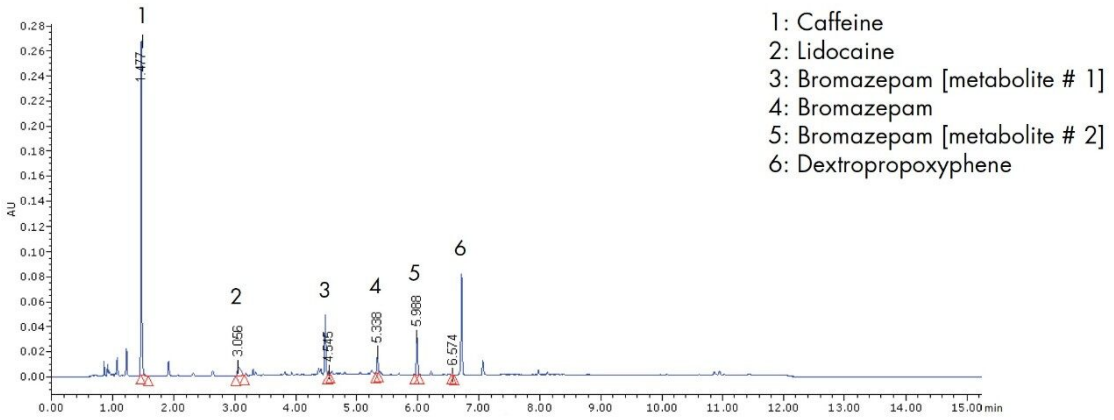

Figure 3. Toxicology screening chromatogram of the same urine sample as shown in Figure2, with UPLC/UV analysis.

Example 2: Toxicology Screening (Urine)

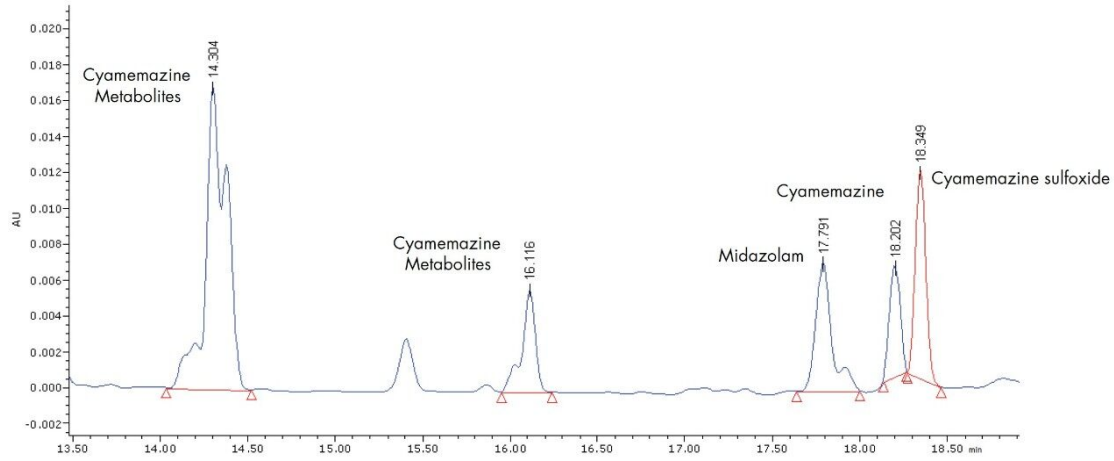

Figure 4. Toxicology screening with HPLC-UV analysis.

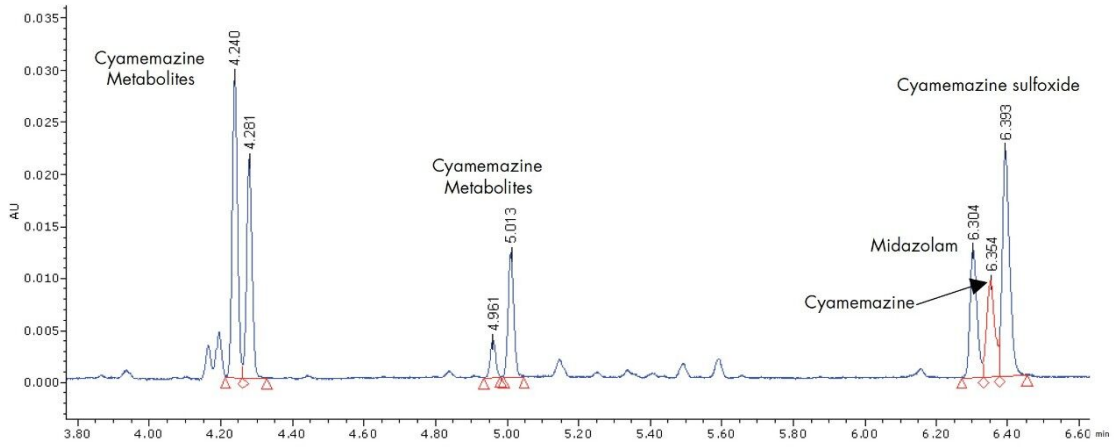

Figure 5. Toxicology screening chromatogram of the same sample as shown in Figure 4., with UPLC/UV analysis.

The rapid calculation of all the chromatographic data parameters is performed automatically by the Empower 2 software,[3] and some comparative performance data between HPLC and UPLC analyses is detailed in Table 1. Solvent consumption is also significantly reduced as a direct result of shorter analytical runs.

	HPLC/UV	UPLC/UV
Run time (min)	40	15
Retention time of the last eluting compound (prazepam) (min)	25.57	9.17
Peak capacity	95	244
Average peak width (min)	0.497	0.087
Separation data		
Resolution:		
chlordiazepoxide - oxazepam	3.7	1.9
clobazam – diazepam	2.7	8.4
clotiazepam - prazepam	2.9	8.4
Peak symmetry :		
amisulpride	1.1	1.0
diazepam	1.1	1.0

Table 1. Comparison of relative chromatographic parameters between HPLC and UPLC.

Superior chromatographic separation minimizes the risk of contamination of the UV spectra, and consequently improves the quality and accuracy of the library match. Reproducibility of the retention time is further improved (Table 2.) and it is possible to arrow the retention time window for increased accuracy in peak identification.

	Repeatability (n=10; successive injections of Test Mixture)		Reproducibility (n=12; injections of Test Mix, 3 per month for 4 months)	
	CV (%) RT	CV (%) RRT	CV (%) RT	CV (%) RRT
Theophylline	0.9	0.7	7.0	4.5
Paracetamol	0.8	0.5	5.5	3.1
Caffeine	1.5	1.2	10.7	8.1
Mirtazapine	0.7	0.6	3.9	1.4
Venlafaxine	0.7	0.6	3.5	1.0
Flurazepam	0.4	0.0	2.6	0.0
Chlordiazepoxide	0.5	0.7	2.6	0.8
Oxazepam	0.5	0.6	2.4	0.4
Imipramine	0.4	0.2	2.3	0.4
Clobazam	0.5	0.3	2.0	0.7
Diazepam	0.5	0.3	1.9	0.9
Trifluoperazine	0.4	0.3	1.8	0.9
Clotiazepam	0.5	0.4	1.7	1.0
Prazepam	0.6	0.5	1.6	1.2
Mean CV (%)	0.6	0.5	3.5	1.7

Key: CV(%) : Coefficient of Variation (%), RT: Retention Time, RRT : Relative Retention Time

Table 2. Repeatability and Reproducibility of Chromatography by UPLC.

The UV library presently contains data for 612 unique compounds including many drug metabolites. It is however, easy to extend or customize this list by injecting pure compound(s) and measuring the relevant UPLC retention times and their corresponding UV spectra.

This type of UV based library approach is popular in many hospitaltoxicology laboratories, as it provides comprehensive screening capabilities for a very competitive cost per sample. The sample preparation method described is extremely versatile and can be used for many different sample matrices. The UPLC

column described has been found to remain in excellent condition after many thousands of separate sample injections.

Laboratory performance with (external) proficiency sample analyses, has shown the method and developed library offer comprehensive, accurate and rapid compound identification.

As the UPLC separation conditions are also compatible with Mass Spectrometry (MS) detection, the UPLC/UV system can act as an ideal entry level solution. The addition of an MS detector when used in line with the UV detector, provides additional confirmatory data that may be useful for accurate quantitation of compounds present at low levels or that that might be necessary for some types of challenging or accredited and legally/regulated forensic analyses.

Conclusion

This application shows that toxicology screening is greatly improved by transferring a traditional HPLC-UV method to UPLC-UV.

The UPLC-UV screening method can be considered cost effective in respect to the initial capital expense when compared to some MS based screening methods. The potential for simpler quantitative or semiquantitative method implementation is also feasible with the system described.

In combination with a sixty percent reduction in analytical run time, both the frequency and accuracy of compound identification is increased. The method has been used on a routine basis for more than one year, successfully replacing and enhancing an existing HPLC system.

References

1. Y. Gaillard and G. Pepin. *Journal of Chromatography A*, Volume 763, Issues 1–2, 28 February 1997, Pages 149–163.
2. Waters Brochure “ACQUITY UPLC Photodiode Array Detector”, ref: 720001332EN.
3. Waters Brochure: Empower 2 Software, ref: 720001527EN.

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