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應用手冊

Analysis of Beta-Blockers Using UPLC with Accurate-Mass Screening

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For forensic toxicology use only.

Abstract

This application note demonstrates the sensitivity and selectivity of the Forensic Toxicology Application Solution with UNIFI in providing comprehensive screening of beta-blockers at low levels of concentration in human urine and achieving the MRPL with minimal sample preparation.

Benefits

- Enables analysts to confidently screen and identify beta-blocker drugs in urine
- Data is processed automatically and presented to the user with fully customizable workflows and reports.

Introduction

Propranolol was the first, clinically successful beta-blocker. Synthesized by JW Black in the early 1960s,[1] it revolutionized the management of angina pectoris and spawned the development of additional beta-blockers. Beta-blockers competitively block the action of beta-adrenergic agonists at the beta-receptors in the cells of heart muscle and other tissues of the sympathetic nervous system. They are legally prescribed and used primarily for the management of hypertension, angina, and cardiac arrhythmias. These substances however, can be abused by athletes who want to decrease their heart rate, lower their blood pressure, or improve their fine motor skills. Consequently, the World Anti-Doping Agency (WADA) includes beta-blockers in its 2014 Prohibited List[2] (Category P2), limiting the prohibition to sports like archery, golf, and shooting.

Recent advances in liquid chromatography and mass spectrometry can help determine the presence of beta-blockers in urine.

Experimental

Sample preparation

A mixed, methanolic standard containing the following beta-blockers was prepared at a concentration of 50 µg/mL: acebutolol, alprenolol, atenolol, bunolol, bisoprolol, carazolol, celiprolol, levobunolol, metipranolol, metoprolol, nadolol, nebivolol, oxprenolol, pindolol, sotalol, and timolol. Blank human urine was spiked with the mixed standard, resulting in final concentrations of 50, 100*, 250, and 500 ng/mL. A simple five-fold dilution with mobile phase A was used to prepare each spiked urine sample for injection.

* Minimum required performance level (MRPL) for a WADA-accredited laboratory.

Method conditions

LC conditions

LC system:	ACQUITY UPLC I-Class (FTN)
Run time:	15 min
Column:	ACQUITY UPLC HSS C_{18} 2.1 x 150 mm, 1.8 µm
Vials:	Waters Maximum Recovery Vials
Column temp.:	50 °C
Sample temp.:	10 °C
Injection vol.:	10 µL
Flow rate:	0.4 mL/min
Mobile phase A:	5 mM aqueous ammonium formate, adjusted to pH 3.0
Mobile phase B:	Acetonitrile with 0.1% formic acid
Gradient:	87% A to 50% A over 10 min, reduce to 5% A and hold for 1.5 min before returning to 87% A

MS^E conditions

MS system:	Xevo G2-S QTof

MS^E conditions

Ionization mode:	ESI+
Source temp.:	150 °C
Desolvation temp.:	400 °C
Desolvation gas:	800 L/h
Reference mass:	Leucine enkephalin $[M+H]^+$ = 556.2766
Acquisition range:	*m/z* 50–1000
Scan time:	0.1 s
Capillary voltage:	0.8 kV
Cone voltage:	25 V
Collision energy:	Function 1: 6 eV Function 2: ramped 10 to 40 eV

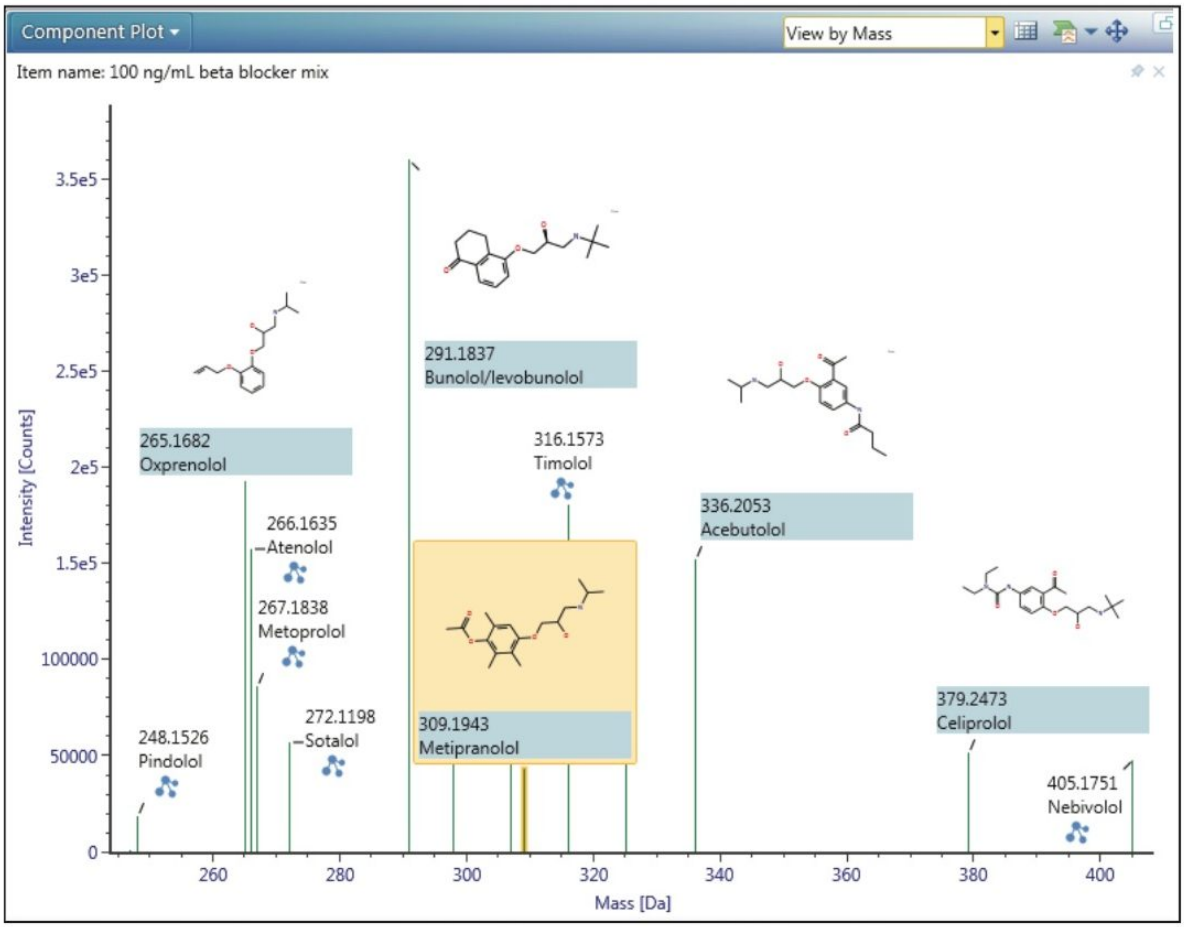
Results and Discussion

The diluted spiked urine samples were injected and data was acquired using the standard MS^E -based toxicology screen.[3,4] Data were subsequently processed using the UNIFI Forensic Toxicology Library comprising more than 1,000 toxicologically-relevant substances. Qualitative identification was achieved through a combination of mass accuracy, retention time (RT) and the presence/absence of expected fragment ions. In the same processing step, UNIFI Scientific Information System also generates and displays any quantitative data.

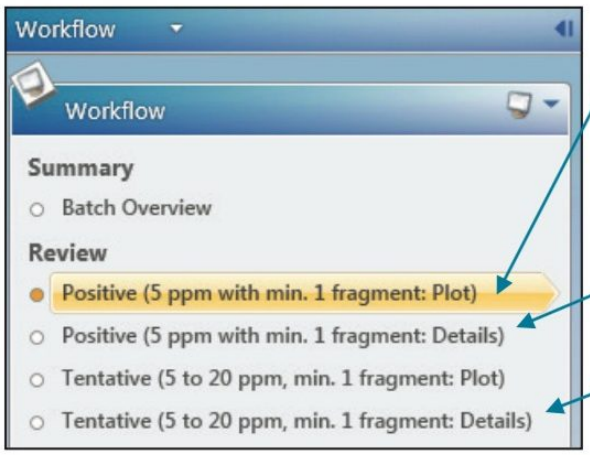
UNIFI uses a simple workflow approach to guide the user through the sample results; data is automatically filtered and presented to the user according to the degree of confidence in the identification, thereby decreasing

the requirement for analyst's review. Workflows are fully customizable - an example of the criteria that may be used is shown in Figure 1.

All of the beta-blockers were successfully identified at the lowest concentration investigated in this study (50 ng/mL) and met the user-defined criteria for a "Positive" drug finding. Figures 1 through 4 provide an illustrative example of some of the data that is automatically-displayed or available to the user on a "single-click" from the Review pane.



"Positive": Component Plot (a graphical display of components which satisfy predefined criteria).



"Positive" criteria: Precursor ion ≤ 5ppm, ≥ 1 expected fragment detected, within 0.35 min of reference RT.

"Tentative" criteria: Precursor ion accuracy 5-20 ppm, ≥ 1 expected fragment ion detected, response >1000, within 0.35 min of RT.

Figure 1. Example of a workflow based on two differing categories of identification ("Positive" and "Tentative") together with a summary of the criteria used for each.

In contrast to the plot view, which provides a very simple graphical display of detected components, full details of each identification can be viewed by selection of the Component Summary (Figure 2). This is a user-friendly table that summarizes key characteristics of identified peaks including mass accuracy, confirmatory fragment

ions and isotope information (in this example, only those components that matched the “Positive” criteria are shown).

The extracted mass chromatograms for the precursor ion and all of the high collision energy fragment ions for a particular component can also be displayed if required, as shown in the Chromatograms window (lower left of Figure 2).

Further information is available by viewing the low and high energy spectra for a component as shown in the Spectra window. This view highlights the precursor ion in the top trace and the found fragment ions in the bottom trace. UNIFI provides improved three-dimensional (3D) chromatographic peak detection with its integrated ApexTrack algorithm, which facilitates the generation of cleaner mass spectra, enabling better library matching of fragment ions.

In addition to viewing the spectra, it is often useful to display a summary of the confirmatory fragment ion data. Figure 2 also shows the Fragments table which contains details for the expected fragments for acebutolol, the mass error associated with each detected fragment, and the detected fragment intensity.

Component Summary: User-friendly table that summarizes key characteristics of identified peaks including information related to: mass accuracy; RT; presence of confirmatory fragment ions and isotope information.

Figure 2. Details for a 50 ng/mL spiked urine sample. The Component Summary shows details for the first five analytes present in alphabetical order. The F v E (%) column displays the number of found vs number of expected fragments, expressed as a percentage. The Isotope Match Intensity RMS Percent and Isotope Match Mz RMS PPM indicate the degree of matching between the theoretical isotopic pattern and the observed pattern for the precursor ion cluster. The chromatogram's window contains the extracted mass chromatograms for the selected precursor i.e., acebutolol (m/z 337.212) and fragment ions (m/z 116, 319, 98, and 260). The Spectra window displays the low collision energy (upper spectrum) and high collision energy (lower spectrum) for acebutolol and the Fragments table shows each of the expected fragments for acebutolol.

The isotopic pattern obtained for each component can also be an aid in identification. Figure 3 shows the mass spectrum of the low collision energy trace for sotalol, a sulphur-containing compound. The two most abundant stable isotopes of sulphur are ^{32}S and ^{34}S which are present at a ratio of 95:4. An algorithm within UNIFI can be used to indicate the degree of matching between the theoretical and observed isotopic patterns for a component, with a low score indicating a good match. This "Isotope Match Intensity RMS Percent" column can be added to the Component Summary table as an extra point of confirmation. A further UNIFI algorithm is used to evaluate the level of agreement between the expected *m/z* and found *m/z* of each isotopic peak and these results are shown in the Isotope Match Mz RMS PPM, again with a low score indicating a good match as shown in the rightmost columns of the Component Summary window of Figure 2.

Isotopic information:
UNIFI includes both a graphical display as well as calculated comparisons of measured isotopic data against the theoretical (see Figure 2).

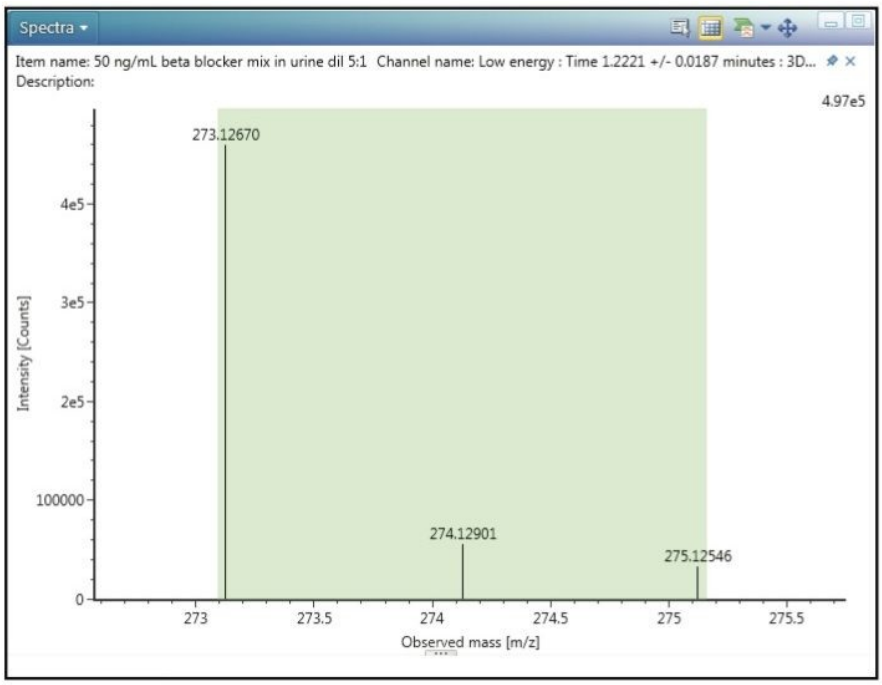


Figure 3. The low collision energy spectrum for sotalol showing the m/z 273 and 275 ions corresponding to the sulphur isotopes 32S and 34S. UNIFI includes algorithms to automatically compare the isotopic data of the measured component with the theoretical for the proposed substance; this data is included in the last two columns of the Component Summary table.

In particular, Figure 4 shows, for each beta-blocker, a semi-quantitative calibration plot that draws data from three replicate injections made at each concentration (50, 100, 250, and 500 ng/mL). The calibrations are calculated from the response value for each analyte, a value that originates with the 3D integration of the monoisotopic precursor-ion peak. Because no internal standards were used in this study, this semi-quantitative data demonstrates only the typical dynamic range of the instrument.

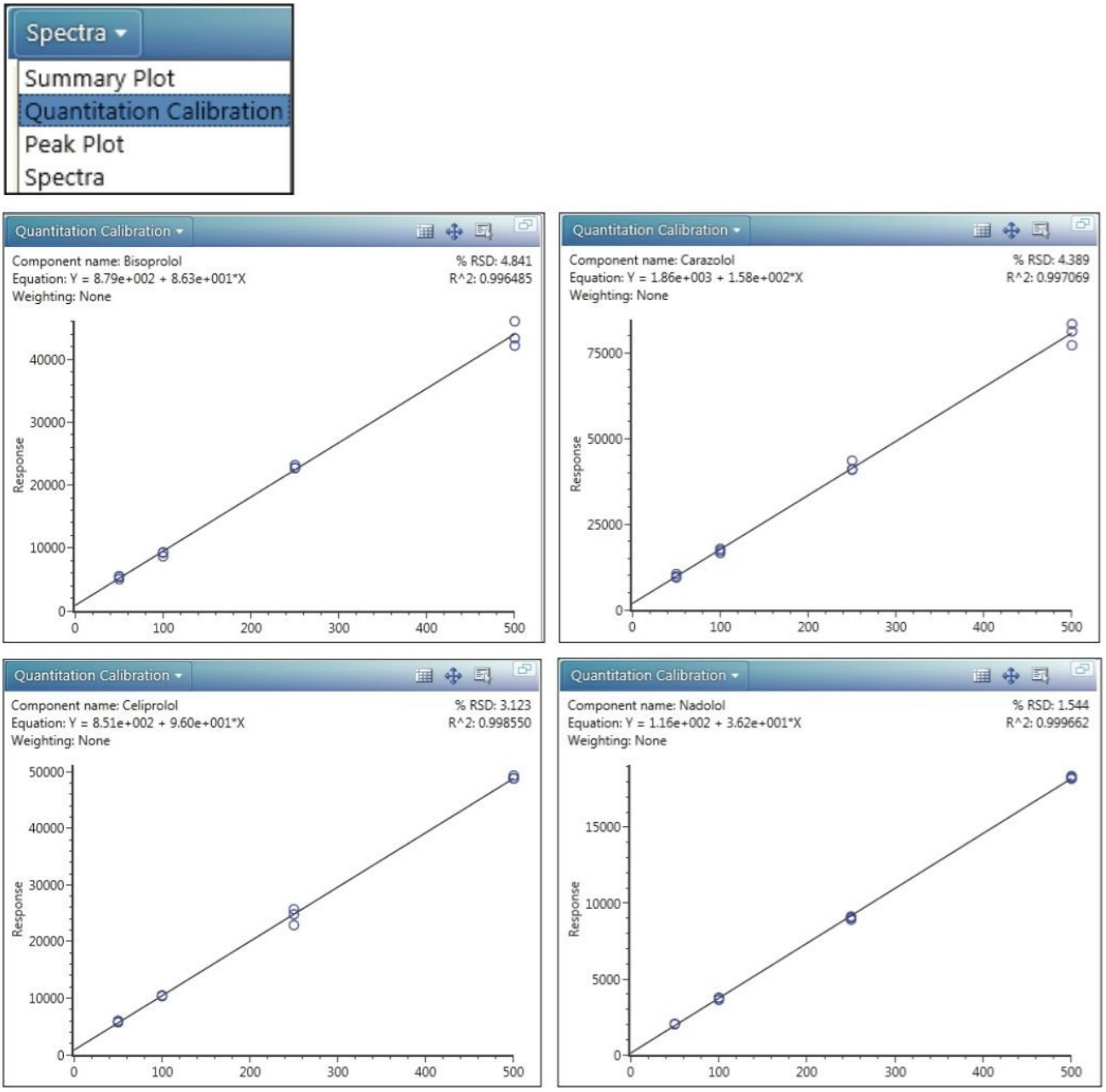


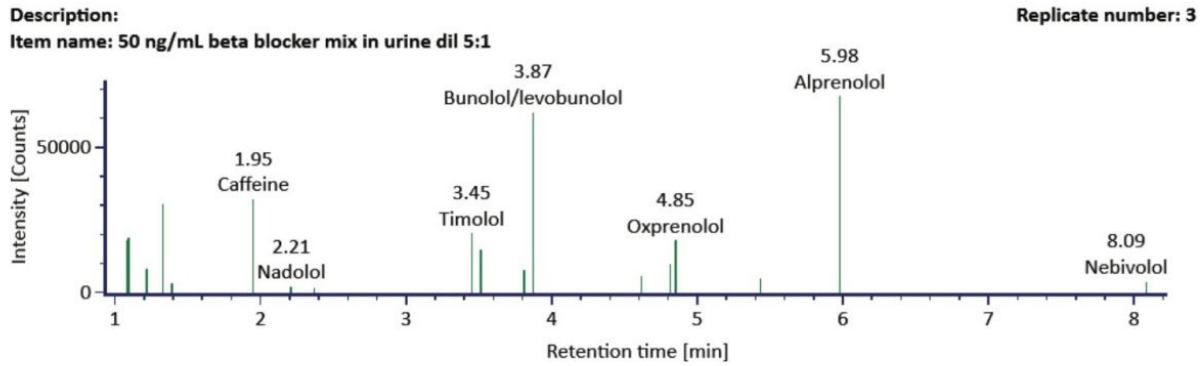
Figure 4. Calibration plots for four beta-blocker analytes spiked into urine (triplicate injections) at 50, 100, 250, and 500 ng/mL using a linear fit with no weighting applied.

Figure 5 illustrates a fully customizable report generated by the UNIFI Software from the results that provided the key details of the identifications made for this sample. A section from this report is shown in Figure 5 and provides a Component Plot as well as a Component Summary for each identification category.

Sample Information

Item name:	50 ng/mL beta blocker mix in urine dil 5:1	Sample type:	
Description:		Replicate number:	3
		Sample position:	1:B,6

Positive Identifications (Mass error <5ppm, at least one diagnostic fragment ion)



Item name: 50 ng/mL beta blocker mix in urine dil 5:1

Component name	m/z	Mass error (ppm)	Expected RT (min)	Observed RT (min)	Identified High Energy Fragments	Expected Fragments Count	F v E (%)	Response	Isotope Match Mz RMS PPM	Isotope Match Intensity RMS Percent
Acebutolol	337.2121	-0.27	3.7	3.5	4	4	100.00	14684	0.48	1.84
Alprenolol	250.1804	1.07	6.2	6.0	2	3	66.00	67513	0.99	1.50

Figure 5. A fully customizable report showing the Component Plot and the first two lines of the Component Summary for this injection.

Conclusion

This application note demonstrates the sensitivity and selectivity of the Forensic Toxicology Application Solution with UNIFI in providing comprehensive screening of beta-blockers at low levels of concentration in human urine and achieving the MRPL with minimal sample preparation. Despite the complex nature of accurate mass MS^E data, the UNIFI Software enables user-friendly, comprehensive data analysis, interpretation, and reporting. The excellent linear dynamic range of this system is demonstrated in four, simple, automatically generated calibration plots.

References

1. Black JW, Crowther AF, Shanks RG, Smith LH, and Dornhurst AC. A new adrenergic beta-receptor antagonist.

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2. The World Anti-Doping Code: The 2014 Prohibited List, International Standard. http://list.wada-ama.org/wp-content/uploads/2013/11/2014-Prohibited-List-ENGLISH-FINAL.pdf (accessed 20 September 2014).

3. Rosano TG, Wood M, Ihenetu K, and Swift TA. Drug screening in medical examiner casework by high resolution mass spectrometry (UPLC-MS^{E}-TOF). *J Anal Toxicol*. 2013 Oct;37(8):580–93. doi: 10.1093/jat/bkt071.

4. Forensic Toxicology Screening. Waters Brochure 720004830EN.

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